Nasal colonization with methicillin-resistant *Staphylococcus aureus* in military personnel in a developing country - Development of a skin and soft tissue infection surveillance system in the Peruvian Air Force

by

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DEDICATION

I dedicate this work to my family who supported me and helped me overcome all the issues that arose during the development of this research. My wife Olga and son Joan for their love, dedication and patience that eased the difficult times I had to overcome and also motivated me to continue working on the research; my mother Etelvina, my father Alberto, my sister Claudia and my aunt Juana for all their encouragement, love and care. To all of them, my infinite gratitude.

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May 15, 2015

ABSTRACT

Nasal Colonization with methicillin-resistant *Staphylococcus aureus* in military personnel in a developing country - Development of a skin and soft tissue infection surveillance system in the Peruvian Air Force:

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This research had two primary objectives. First, to determine the natural history of nasal colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) among military personnel in a developing country; and second, to develop an epidemiological surveillance process for skin and soft tissue infections (SSTIs) in the Peruvian Air Force through the implementation of reporting of individual events within the current electronic disease surveillance system.

For the first objective, we conducted a prospective cohort study at the four largest bases of the Peruvian Air Force, collecting nasal swabs from 756 active duty military personnel. The samples were cultured to identify the presence of *Staphylococcus aureus*, and the antimicrobial resistance profile was then assessed. All methicillin-resistant *Staphylococcus aureus* isolates were identified and genotyped at USUHS laboratories to

identify the molecular profile of the isolate. For the second objective, we implemented surveillance of skin and soft tissue infections (SSTIs) in the current electronic surveillance system of the Peruvian Air Force, and after 9 months, we evaluated the performance of the SSTIs surveillance system implemented at the 27 health facilities of the Peruvian Air Force.

Our findings demonstrated that nasal colonization with *Staphylococcus aureus* was lower than expected at baseline, but increased over the study period to levels consistent with published rates in the region. To our knowledge, this study that assessed rates of nasal colonization at military bases in four different cities in Peru was the first study of MRSA in military population in Latin America. A MRSA strain (similar to New York/Japan MRSA strain) not previously reported in Peru was found to be circulating in the country. Risk factors for acquisition of *Staphylococcus aureus* (SA) in this population were consistent with those reported in the literature. Regarding the SSTI surveillance, many factors affected the performance of the SSTI surveillance system. We identified numerous challenges to implementing disease surveillance systems within a developing setting.

In summary, our study found a low prevalence of baseline nasal colonization with *Staphylococcus aureus* (9.7%) and MRSA (0.3%) in an active duty military population in Peru. However, the prevalence increased over the study period to 20.4%; we also identified a non-typical community-associated MRSA strain circulating in Arequipa, different from those previously described in the country; and we implemented a SSTI surveillance system with the Peruvian Air Force, but identified several challenges for the adequate functioning of a SSTI electronic surveillance system.

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CHAPTER 1: Introduction

The purpose of this research project was to increase the current knowledge about nasal colonization with *Staphylococcus aureus*, to identify possible resistant strains (MRSA), to assess the rates of nasal colonization with these strains, and to provide a framework to improve the surveillance of the main clinical event related to infection with community-associated MRSA in active duty military population of the Peruvian Air Force.

ANTIMICROBIAL RESISTANCE: A GLOBAL PROBLEM AMONG *STAPHYLOCOCCUS AUREUS* AND OTHER BACTERIA

Antibiotic resistance is one of the greatest threats to the global health since the development of the first antibiotics. Unfortunately as new antibiotics were discovered, bacteria adapted and started developing resistance to these medications as a natural mechanism of defense. The indiscriminate use of antimicrobials worldwide, the uncontrolled availability and bad prescription practices led to the rapid development and spread of antimicrobial resistance, which is now a global phenomenon. These antimicrobial resistant bacteria were not restricted to healthcare settings, but found in the community as well. The main consequences of the antimicrobial resistance are that it impairs effective treatment, increases costs associated to medical care, put patients infected with resistant microorganisms at risk for adverse outcomes, and accelerates microbial evolution in unpredictable ways.

This worldwide phenomenon of the increase in antimicrobial resistant bacteria requires prompt and decisive action in order for it to be contained. The World Health Organization (WHO) established six measures to combat antimicrobial resistance. These

steps require the actions of different actors to obtain a better control of this threat. The steps include the development of national action plans, improvement in the surveillance and laboratory capacity to improve disease surveillance, the insurance of access to adequate medicines, the promotion of the rational use of antibiotics and adequate medical care for improved infection control and prevention (IPC), and encouragement of research and development in this field (53). The application of these recommendations has been varied, and in developing countries, the lack of consistent and accurate information regarding antibiotic resistance makes this task difficult to accomplish. One bacterium, which developed resistance in the early days of the antimicrobial era, is *Staphylococcus aureus* and currently multiple resistant strains are circulating worldwide. Studies have found that MRSA exposure was no longer restricted to healthcare settings, but was present in the community as well.

The increasing prevalence of antibiotic resistant strains of *Staphylococcus aureus* is now a global problem with MRSA being the classic culprit that affects military and non-military populations around the world. By 2013 the Centers for Disease Control and Prevention's (CDC) report about antibiotic resistance listed MRSA as one of the most serious threats causing 80,461 severe infections and 11,285 deaths per year in the U.S., leading to a heavy burden of the healthcare system (17). Consistent with the CDC report, the 2014 WHO Global Health Report on Antimicrobial Resistance, which used information from published reports and sentinel reference labs around the world, reported that in all the WHO regions, MRSA prevalence was above 20% and that being infected with a MRSA strain increased the risk of death and the associated cost of healthcare, hospitalization, and antibiotic therapy (54). These study findings were similar to those

found in a simulation by Lee et al. (2013) about the economic costs of infections with community-associated MRSA (CA-MRSA) in the US, which concluded that the costs were overwhelming for society; and specifically for the US Army. The annual total costs ranged between 14 to 32 US million dollars (38). There is increasing knowledge about MRSA in the developed world but a marked absence of data from developing country settings. The 2014 WHO report included a systematic review of the evidence related to the economic and health burden of MRSA. Of 147 studies included, almost 80% presented data from high-income counties, and the other 20% came from upper-middle countries. None of the studies came from low-income countries. From Latin America, there were seven studies included in the review, three from Brazil, two from Colombia, one from Mexico and another from Argentina, all in hospitalized patients.

The consequences of the spread of antimicrobial resistance have deep impacts on healthcare, including the use of less effective antibiotics and hospitalization in order to treat infections that in the past only required outpatient treatment; raising the costs of care as well as the morbidity and mortality. This was the first study to systematically determine the prevalence and the molecular characteristics of MRSA among Peruvian active duty military population in multiple cities in the country.

Genetic and physiological characteristics of *Staphylococcus aureus*

Staphylococcus aureus are commensal Gram-positive bacteria usually found on the skin and mucosa of humans. *Staphylococcus aureus* are the etiologic agents for multiple infections of different organs and systems of the human body, ranging in severity from mild to life threatening. Unfortunately *Staphylococcus aureus* also has the capability to develop antimicrobial resistance in a rapid fashion. Two parts of the

Staphylococcus aureus genome include: (1) a stable core genome that encodes proteins related to the control of metabolism, replication, and virulence factors; and (2) an accessory and more flexible genome which encodes proteins easing *Staphylococcus aureus* adaptation to different niches and environments (30; 42). This accessory genome was acquired through the transfer of mobile genetic elements (MGEs). The *Staphylococcus aureus* bacterium possesses many types of MGEs including the staphylococcal cassette chromosomes (SCCs). These SCCs are large fragments of DNA and encode for antibiotic resistance or virulence determinants (42). The virulence of this strain is determined by the expression of virulence factors involved in the adhesion and invasion of host cells, the degradation for the host cell, evasion of immune response; and use of nutrients in the host (30). Successful colonization and invasion of the host is dependent also on the evasion of the immune system. There are four mechanisms involved in the evasion of the immune system: (1) formation of biofilms by the excretion of exopolymers or capsular polysaccharide that prevent recognition; (2) inhibition of receptor-mediated recognition by neutrophils and other phagocytes. *Staphylococcus aureus* produces a chemotaxis inhibitory protein (CHIPS) and also an inhibitor of C3 convertase (SCIN); two molecules that interfere with the recognition by immune cells; (3) impairment of phagocytosis by the expression of catalase and superoxide dismutase and other molecules; and finally by (4) the use of active mechanisms that attack directly the host immune cells like hemolysins and the expression of super antigens that interfere with the immune response against the bacterium. These mechanisms of avoiding immune response have been reported to be enhanced in CA-MRSA (21).

Definition of MRSA

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first documented in 1960, after the introduction of the semi-synthetic penicillins. Until the late 1990s, its presence was confined largely to hospital settings (HA-MRSA) with occasional outbreaks. Since the 1990's the number of outbreaks and infections caused by MRSA, specifically community –associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) increased steadily (20; 76); The differences between these two main epidemiological subtypes of MRSA: hospital-associated (HA-MRSA) and community-associated (CA-MRSA) are defined by: the genetic composition that determines the antibiotic resistance, the characteristics of the affected population, and the clinical disease manifestations. Both strains contain mobile genetic elements that confer antimicrobial resistance to beta-lactam antibiotics. In the case of HA-MRSA, this resistance is acquired by the presence of the large Staphylococcal cassette chromosomes (SCCmecs I – III), which provides resistance to both beta-lactam and many non-beta-lactam antibiotics (multi-drug resistant) (20). On the other hand, CA-MRSA typically possesses SCCmec type IV that only confers resistance to beta-lactam antibiotics. Although less resistant to antimicrobials, CA-MRSA possesses other elements that increase its pathogenicity and virulence. These virulence determinants include: Panton-Valentine Leukocidin (PVL), the arginine catabolic mobile element (ACME), and over expression of α-hemolysin (42).

PVL is an exotoxin that forms pores in the membranes of cells, destroys leukocytes through cytolysis and also showed dermonecrotic action in some animal models. ACME encodes proteins that facilitates the colonization of the skin and also improves its fitness, (adaptation to the environment), and it is only found in USA300

strains. The α-hemolysin also forms pores in the cell membrane. Other virulence factors identified include alpha-toxin which lyses immune cells other than neutrophils like macrophages and lymphocytes; phenol soluble modulines (PSMs) which promote inflammation and also cytolysis; protein A that prevents opsonization and affects the cellular immune response (7; 20; 21; 42; 55).

Classification of MRSA strains

There are different ways to classify MRSA strains. Based on the presence of SCCmecs, there are nine types (I to VIII and V_T) of MRSA strains (20). However, multiple typing methods have been used and most of the strains responsible for outbreaks have been characterized using pulsed-field gel electrophoresis (PFGE). The CDC uses this technique to classify the *Staphylococcus aureus* isolates in the USA according to the PGFE patterns identifying twelve patterns (USA100 – USA1200) (46; 72). Other methods for typing MRSA strains are amplified fragment length polymorphism (AFLP) and multilocus sequence typing (MLST) which assign other names to the strains and are used in other parts of the world (46; 62). The association between the different methods of characterization is reviewed in Table 1.

The USA300 genotype has become the predominant CA-MRSA strain recovered from outbreak investigations in USA; and also has been isolated in different places around the world. Nevertheless, instead of being a unique strain, USA300 apparently is a family of strains that possess more than 80% similarity within the PFGE patterns (46). This USA300 strain carries SCC*mec* type IV, PVL genes, and the ACME element, is resistant to betalactam drugs, is frequently resistant to erythromycin, and is found predominantly on community associated skin infections. This strain is the most

commonly found in US soldiers and was the most common cause of skin and soft tissue infections (SSTIs) in this population (20; 24; 47; 55; 71).

USA300, the most common cause of CA-MRSA infections, is distributed worldwide and has been reported in different countries in Europe and Asia, such as Japan (72). Mendes et al. in 2010 analyzed MRSA isolates from Europe and the Americas in terms of antimicrobial susceptibility and genotyping. They found a different distribution in the strains of MRSA around the world. For example, USA300 is the most common strain in the US. This strain possesses more susceptibility to clindamycin and fluoroquinolones than other strains found around the world (like those isolated in Russia [ST5], or in South America and Europe [ST8]); but most of the strains analyzed "belong to the same clonal lineage" and share similar characteristics in terms of antimicrobial susceptibility (49). This has implications for treatment, especially in deployed military personnel taking into account that previous use of antibiotics plays a role in CA-MRSA colonization. These findings correlate with the fact that USA300 is itself comprised of different strains with slight differences in the genome sequence. There appears to be slight variations among the MRSA strains worldwide (specifically USA300) although many of them are genetically related (20; 52). Apparently MRSA has evolved multiple times, acquiring multiple SCCmec in different strains. Therefore, the rapid transmission of mobile genetic elements between different strains of CA-MRSA may contribute to the broad dissemination of some strains after contact between local strains and imported strains. It is suspected that the reservoir for the SCCmec type IV are methicillin-resistant coagulase negative-staphylococci and there was a horizontal transmission among this

species and *Staphylococcus aureus*, leading to the development of CA-MRSA strains (50).

Table 1. Comparison between the different types of classification of MRSA strains

MLST*	**PFGE***	**SCCmec***	**Published Name**[§]
ST1	USA400	IV	
ST5	USA100	II	New York/Japan
ST5		I	Cordobes/Chilean
ST5	USA800	IV	Pediatric
ST8	USA300	IV	
ST8	USA500	II, IV	
ST8		I, III, IV	Archaic/Iberian
ST8			
ST22		IV	
ST30	USA1100	IV	Mexican clone
ST36	USA200	II	
ST45	USA600	II	
ST45		IV	
ST72	USA700	IV	
ST239		III	Brazilian/Hungarian
ST240		III	Brazilian/Hungarian
ST247		I	Archaic/Iberian
ST250		I	Archaic/Iberian

*MLST: Multilocus sequence typing, PFGE: pulsed-field gel electrophoresis, SCCmec: Staphylococcal chromosome cassete

[§] Published names compiled by Deurenberg, R. and Stobberingh, E. (2008)

Natural history of nasal colonization by *Staphylococcus aureus*

The nares are the primary site of colonization for *Staphylococcus aureus*. The colonization rate varies within populations: 20%-30% are colonized persistently, 20% have an intermittent pattern of colonization and 50% are never colonized (5; 23; 26). The definition of the categories of carrier status is usually made based on the number of positive swabs/number of total swabs for each person. These three categories were assessed based on longitudinal studies that looked for the recovery of the baseline isolates

after different periods of time, usually each week or month during 6 months to 1 year (25; 26). The usual methods for collecting samples from the nares involve the use of sterile nasal swabs with charcoal to increase the recovery of *Staphylococcus aureus*, its transportation and storage under refrigerated conditions and its culture in enriched broth. Eriksen et al. (1994) demonstrated that the use of different lab procedures (type of swab, use of enriched media, and time for processing) did not affect the rate of recovery of *Staphylococcus aureus* in nasal swabs in permanent and persistent carriers (25). Researchers typically follow the same strict protocols at different labs worldwide that make them reliable across the community; it is possible that the reliability may be adversely affected by a variety of factors associated with the field procedures

The asymptomatic carriage of *Staphylococcus aureus* at the anterior nasal mucosa is considered as the primary natural reservoir of this bacterium. Nasal colonization is facilitated by the anatomy of the nasal vestibule (an area with only cilia and few mucous secretions) and the resistance of *S. aureus* to microbicide peptides in the mucus (78). The duration of asymptomatic colonization is variable. Intermittent carriers may carry different strains over time unlike persistent carriers who have the same strain. The duration of nasal colonization also varies as subjects become older. Based on research by Sollid et al., nasal colonization with *Staphylococcus aureus* starts at birth, decreases during the first five years and then it increases until 50% are carriers between 6 to 12 years; and finally it decreases as children grow up and become adults (67). Frank et al. (2010) analyzed the composition and dynamics of the nasal microbiota of healthy individuals and hospitalized patients and how it correlates with *Staphylococcus aureus* colonization. Three patterns were identified: (1) in healthy individuals, *Actinobacteria*

were predominant; (2) in *S. aureus* non-colonized inpatients, *S. epidermidis* was predominant, reducing *Actinobacteria*; and (3) in *S. aureus* colonized patients, the reduction of *Actinobacteria* and *S. epidermidis* was remarkable (26). Therefore multiple factors must occur to favor the colonization with *S. aureus*. Apparently *S. aureus* colonization requires a change in the microenvironment of the nares mediated by microbial competition, a scenario where the presence of virulence factors likely play an important role (78). Two population based studies in two different cities: Lima and Cajamarca, showed that the prevalence rate of nasal colonization with *Staphylococcus aureus* in children ranges from 24.6% (12) to 40.3% (14).

Colonization of the nares is a risk factor for subsequent infection by *Staphylococcus aureus* (37; 75). Therefore, it should be expected that high rates of infection should match high rates of colonization, as it is observed with HA-MRSA. However, for CA-MRSA, high rates of *S. aureus* infection do not correlate with high prevalence of nasal colonization. Three scenarios could explain this apparent contradiction: (1) CA-MRSA does not require previous colonization; (2) there are other colonization sites for CA-MRSA like the oropharynx, axilla, groin, and perirectal area (5; 20; 21; 76), or (3) the presence of the SCCmecs implies a fitness cost to CA-MRSA that can affect its rate of growth. This also can explain the other transmission routes of this pathogen among the population. Direct contact (skin contact) may be the main route; however, fomites also play a role in closed settings such as those experienced by military personnel and child daycare centers. In the case of military personnel in the US, the cumulative incidence of skin and soft tissue infections after 10 weeks of follow-up at training facilities was 38% in CA-MRSA colonized subjects while in non-colonized it

was only 2% which was similar to that found in subjects colonized by methicillin sensitive *Staphylococcus aureus* (24). Therefore, previous colonization may play a critical role in the development of these skin and soft tissue infections. However, the interaction between the mobile genetic elements and the local microbial environment at the colonization site may also be a significant factor with colonization and development of infections. At this point, the patterns of colonization, the duration of colonization and transmission routes are still unresolved for CA-MRSA.

Risk factors associated with nasal colonization with MRSA strains

Risk factors related to the acquisition of MRSA and the resulting clinical syndromes are different depending on the strain. HA-MRSA is more associated with older persons exposed to healthcare settings (history of hospitalization, surgery, dialysis, long-term care facility residence, previous MRSA isolation). The clinical syndromes associated with HA-MRSA are pneumonia, urinary infection, surgical site infection, blood stream infection, and sepsis(13). On the other hand, CA-MRSA usually involves young healthy people especially in closed environments (athletes, military personnel, and prisoners), the presence of skin disruption, and recent antibiotic exposure. Likewise, CA-MRSA disease manifests most commonly as skin and soft tissue infections (SSTI). King et al., found that 87% of all the MRSA strains isolated from skin and soft tissue infections corresponded to CA-MRSA strains (mostly USA300) (34), while Seybold et al. found that the concurrent SSTI infection by this strain increased the odds of bloodstream infection by almost 4 times (64). CA-MRSA nasal colonization is a risk factor for future infection (66; 70; 78). Although there was no laboratory recovery of CA-MRSA in colonization studies in early outbreak investigations, this could be attributed to the use of

antibiotics in people who were tested during that time period. Also, nasal colonization was correlated with subsequent *S. aureus* bacteremia (24; 75).

Distribution of MRSA strains

MRSA is distributed worldwide and asymptomatic carriers such as travelers may be a source for dissemination. Various studies have shown that travelers may play a role in the spread of MRSA. In a study performed in Sweden among travelers and immigrants, MRSA colonization was associated with travel to Africa and the Middle East, and it was higher among men and in those who traveled for work purposes (69). However, the strains were similar to those acquired at the country of origin. Military deployment is a specialized form of travel. In studies performed in combat hospitals at Iraq, the prevalent MRSA strain was USA300 in US combat soldiers, which may be related to previous colonization prior to deployment; in the non-US patient populations the rates of MRSA were low, so not much information about types was available (15; 51). In the military setting, *Staphylococcus aureus* infections complicate combat-related injuries and produce skin and soft-tissue infections during deployments or training. Risk factors for SSTI in military members include skin microtrauma and change in the pattern of colonization as a consequence of lower levels of hygiene (82). Although deployed service members and travelers appear to be at some risk for SSTI with MRSA, the importance of nasal colonization and the actual risk of infection are not clear. Military personnel are similar to travelers in general because their activities involve multiple deployments, which vary over time and place during their career. This exposure to different environments can increase the acquisition of MRSA (carriage or infection). The colonization by multiple strains of *S. aureus* favors the horizontal transmission of resistance (interchange of

mobile genetic elements that confers resistance among bacteria at the nares), which may lead to the simultaneous presence of sensitive and resistant bacteria of the same species at the nares (discordance in the strain carriers). See Figure 1.

Some infections caused by *Staphylococcus aureus* follow a seasonal pattern. It has been described that skin and soft tissue infections have a peak during the summer in the general population, a fact that was corroborated in the study of the US military population, which showed an increase of cases during the summer from 2002 to 2005. (2) A differential pattern for hospitalizations was reported when Klein et al. analyzed hospitalizations due to MRSA strains, showing a peak during the summer for infections with CA-MRSA, while HA-MRSA infections had a peak during the winter (35). However, the seasonality of nasal colonization varies greatly among the studies reviewed by Leekha et al. in 2012. Of the eight studies assessing nasal colonization, in two of them the authors found an increase in nasal colonization during the summer (Japan) and spring (Sweden); however, the rest did not show seasonal trends for nasal colonization (40).

MRSA in Latin America and Peru

In Latin America, four clones are the most prevalent: Brazilian, Pediatric, Cordobes /Chilean and New York/Japan clones, with marked differences in virulence, antimicrobial resistance profile and geographical distribution (61). These clones can evolve from methicillin-sensitive *S. aureus* (MSSA) to MRSA and vice versa.

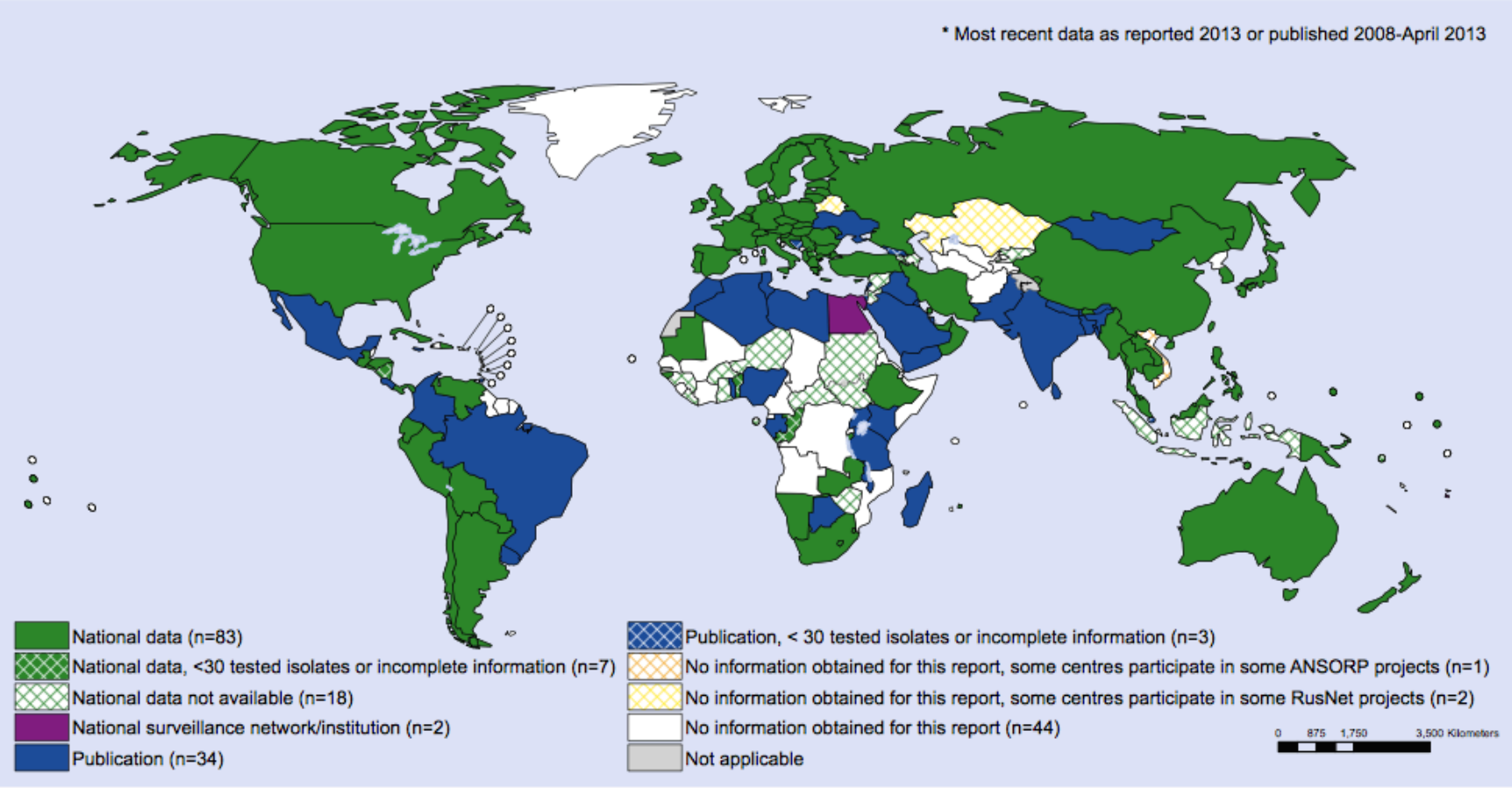


Figure 1. Worldwide distribution of MRSA. Taken from WHO, Antimicrobial Resistance Global Report on Surveillance 2014 (pp. 20)

In Latin America, nosocomial infections are usually due to epidemic hospital associated clones (HA-MRSA), but CA-MRSA is growing and there is a lack of information regarding these CA-MRSA strains circulating in this region. In order to address this issue, a surveillance network for resistant bacterial infections was organized in 1998 under Pan American Health Organization (PAHO) sponsorship; this surveillance system includes specific national and regional hospitals with adequate laboratory infrastructure and resources. However, there is little information regarding the prevalence in other areas, so the geographic extent and the characteristics of MRSA infections in Latin America is not well described (48). The surveillance data is incomplete because it is based mostly on national reference labs from capital cities, which are limited in number and area covered (61).

In 2011, Jones et al. reported the results of the Latin American regional resistance surveillance program; using samples from tertiary-level hospitals from 11 Latin American countries, including Peru. The overall rate of MRSA among *Staphylococcus aureus* isolates was 48%, ranging from 29% (Brazil and Colombia), to 79% (Venezuela) (33). In Brazil, the rates of nasal colonization with *Staphylococcus aureus* was from 9.1% in neonates hospitalized at ICUs while in children from outpatient clinics and day care centers it ranged from 20% to 48%, while the rates of MRSA colonization were 0.6% in neonates, and up to 6% in children (6; 74). Three different MRSA strains were identified: Brazilian, Pediatric (Hospital acquired) and the Western Australian 1/WA1 clone in the outpatient clinic (74).

The epidemiology of MRSA in Peru is not well described. Reports usually are derived from tertiary hospitals in capital cities. For instance, the International Nosocomial Infections Control Consortium (INICC) has collected surveillance data regarding device-associated infections in ICUs at tertiary hospitals from developing

countries since 1998. By 2008, there were 100 hospitals from 29 Latin American countries enrolled in this initiative. Selected Peruvian hospitals started participating in this surveillance network in 2003. Cuéllar et al. reported that the most common agents causing ventilator-associated pneumonia (VAP), central venous catheter-associated bloodstream infection (CVC-BSI), and catheter-associated urinary tract infection (CAUTI) were *Enterobacteriaceae* (25.6%) and *Staphylococcus aureus* (22.2%). Of the *S. aureus* isolates, 73.5% were MRSA but they were not typed (19). García et al. described 3 cases infected with CA-MRSA in Peruvian citizens returning from abroad. The three isolated strains were different: one was the ST30, which was described in Argentina, while the other two belonged to the ST8 clone, but differed in the presence of the arcA gene. The clone ST8 is related to the clone USA300 (27). In another multicenter study that included 32 hospitals from four Latin American countries, clinical samples from different infectious sites (blood, surgical wounds, secretions and complicated SSTIs represented 61% of all the samples), were collected prospectively and their molecular characteristics were assessed. Peru had the highest rate of MRSA isolation (62%) (60).

Current information about MRSA in Peru depends heavily on hospital-based samples from tertiary hospitals with adequate protocols for sampling, collection, isolation, and laboratory infrastructure and resources, with all being limited to case series. Unfortunately, this scenario does not inform us about the presence and dissemination of MRSA in the community and in young and healthy populations, the epidemiological characteristics of this population, the circulating MRSA strains, and their relationship with other illnesses (SSTIs, pneumonia, etc.).

SURVEILLANCE OF SKIN AND SOFT TISSUE INFECTIONS

As stated above, the most common clinical event associated with nasal colonization with MRSA strains is the development of skin and soft tissue infections (SSTIs). These include all the clinical events that affect the layers of the skin (dermis, epidermis, superficial fascia and subcutaneous tissue); based on the depth of the infection we will have different clinical entities including: erysipelas, impetigo and folliculitis (epidermis); ecthyma, furunculosis, and carbunculosis (dermis); cellulitis (superficial fascia); and necrotizing fasciitis (subcutaneous tissue) (59). See Figure 2 and Appendix 6b.

According to the CDC, epidemiological surveillance is defined as "the ongoing systematic collection, analysis and interpretation of health-related data essential to the planning, implementation, and evaluation of public health practice" (16). Any epidemiological surveillance system must establish objectives which can vary from collecting count data to estimate baseline rates of health events, detect outbreaks in real time, generate research hypothesis, monitor changes in the behavior of diseases, etc. Once the objectives are stated, case definitions must be developed, as well as the collection instruments and means for entering the information into the system (this can range from paper-based systems to global real-time online systems). These case definitions and methods must adapt to the type of surveillance that will be implemented: passive when health personnel report in a case-by-case manner, as they are diagnosed; or active, when the health personnel look for the cases trying to capture them before they go to the health center (39).

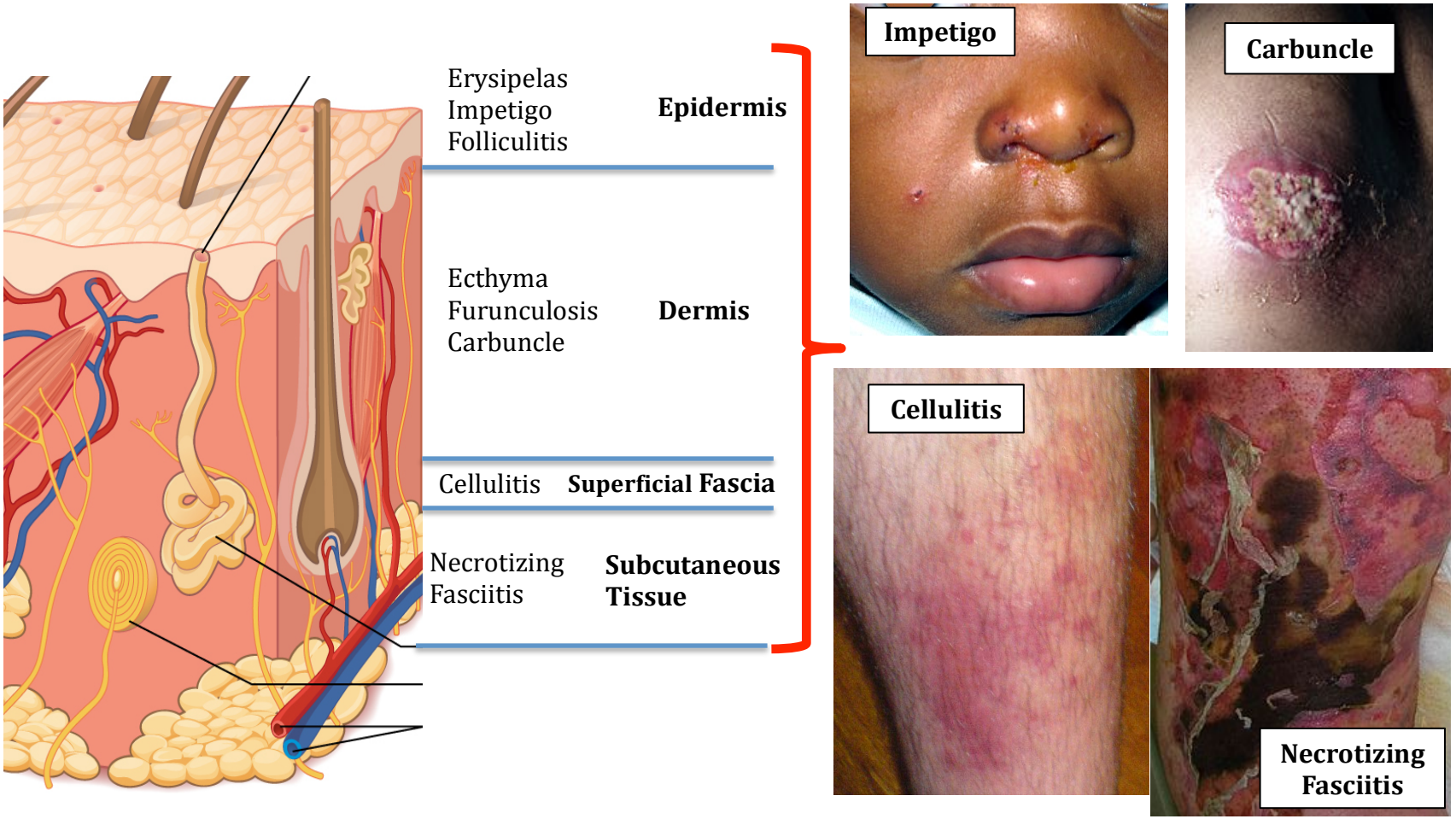


Figure 2. Skin structure and skin and soft tissue infections

In order to be useful, a surveillance system should operate efficiently in order to accomplish the objectives. An effective surveillance system requires continuous monitoring and periodic evaluation in order to ensure that the information collected will be accurate and useful for future decision-making processes in Public Health. Routine monitoring will identify issues that might arise with the different processes involved in epidemiological surveillance (28; 79). The CDC guidelines for evaluating surveillance systems include measures of the events under surveillance and measures of "simplicity, flexibility, data quality, acceptability, sensitivity, predictive value positive, representativeness, timeliness, and stability" (28; 39)). An ideal surveillance system should have a high response rate on all of these attributes in order to be considered effective.

The Peruvian Armed Forces implemented an electronic surveillance system for infectious diseases during the last decade (2004-2010). Information received in reports from each base of each branch is entered into an online database, accessible only with a username and password. Currently, the system receives information on 50 infectious diseases, but skin and soft tissue infections were not included in the system; despite being a clinical event highly associated with a military career.

Before 2009, the Peruvian Air Force only used paper-based forms to collect information regarding diseases in order to assess baseline data on morbidity and mortality. The system consisted of the monthly collection of the ICD-10 diagnoses and the completion of an Excel spreadsheet with aggregated data separated by rank and age. This file was sent monthly to the Unit of Statistics of the Directorate of Health of the Peruvian Air Force. The information about date of diagnosis, onset of symptoms, and prescribed medications were not collected. In 2009, an electronic epidemiological surveillance system

for infectious diseases and injuries was implemented in each base of the Peruvian Air Force. The objectives of the system were to estimate baseline rates for diseases under surveillance, provide early detection of outbreaks, and inform military leadership on public health issues (68). The electronic surveillance system implemented in the Peruvian Air Force has three sequential processes: data collection, data analysis and response. Each process is executed by different stakeholders (See Figure 2). The data collection process starts at each military base where health personnel (called the stakeholders) collect the health information related to the diseases under surveillance. After this, the stakeholder summarizes the data and enters a report into the system. Infectious diseases are considered individual reports, which would be entered into the system within one day after detection. The stakeholder completes a questionnaire either by phone or via a webpage to enter the data into the system with a username and password. After the report is recorded, the second process, data analysis occurs. Designated personnel perform analysis at a central location in Lima, Peru. The analysis includes quality control evaluation (errors detection and duplication of reports), monitoring of reporting activities for each unit, and the analysis of the data with the calculation of rates for infectious diseases, early outbreak detection and writing weekly reports. Reporting activities vary based on the results of surveillance. If an outbreak is detected, an immediate report is made to the relevant health authorities. Other health information captured through the system is reviewed at different intervals by the health authorities (weekly, monthly or quarterly,) depending on the severity of the issue. The administrator sends weekly reminders by phone to stakeholders at each base to ensure an on time report of the events. In case a report was not sent, the stakeholders receive an oral reprimand by the system administrator and if this behavior continues, this situation is

reported to the command of each base to take corrective measures. In the final process, the health authorities develop guidelines and implement control measures in response to reported events.

Lack of complete information regarding SSTIs in the Peruvian military population impairs the design of preventive measures to reduce the burden of these conditions. Presence of SSTIs in a military population can affect the mission readiness. Currently there are 50 infectious diseases under surveillance that require an individual report. However, there has been NO information regarding skin and soft tissue infections (SSTIs). In the Peruvian Air Force, the prevalence rate of SSTIs during the period 2010-2011 was 6.2% based on hospital and clinic records; however, the data came mainly from hospitals in capital cities and the information from operational units without hospitals was not considered. Therefore, there are no reliable rates regarding the burden of SSTIs in military populations, but we nonetheless expect the values to be high among active duty personnel.

Given the lack of consistent information about nasal colonization with *Staphylococcus aureus* and the main clinical event (SSTIs) related to infection with CA-MRSA, this study conducted an assessment of baseline rates of nasal colonization with *Staphylococcus aureus* and MRSA in active duty military population located at 4 urban centers in Peru, the detection of change of nasal colonization status after 6 months and 1 year, and the implementation of a skin and soft tissue infection surveillance system oriented to assess baseline rates of this group of diseases whose etiologic agent is usually *Staphylococcus aureus.*

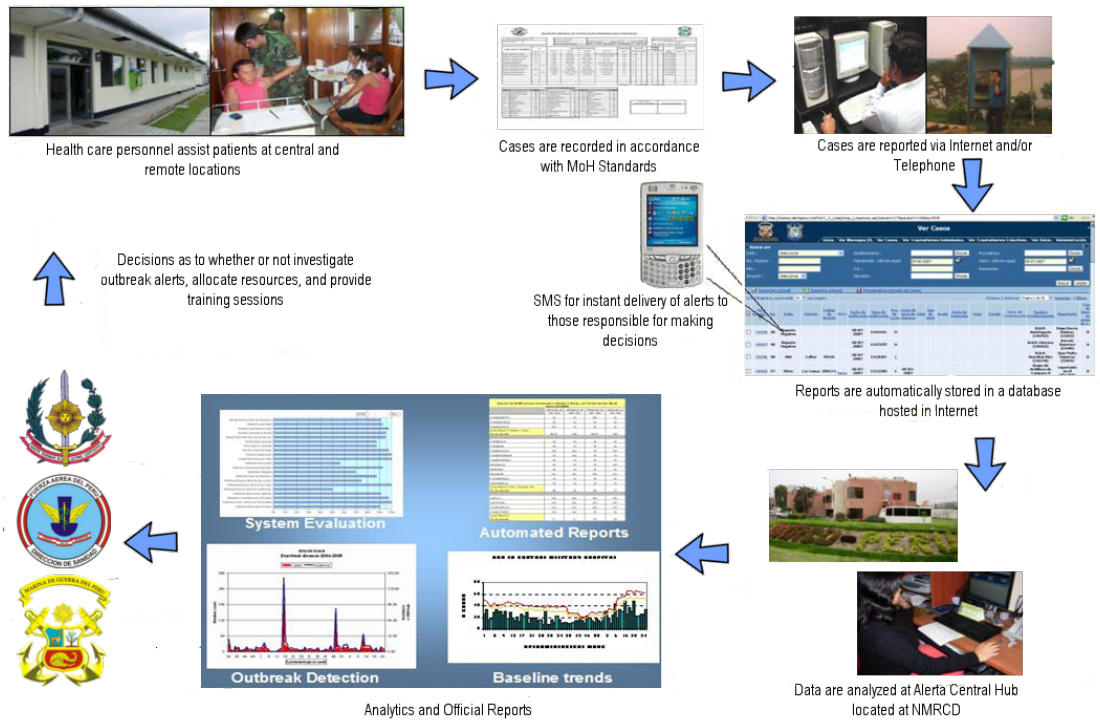


Figure 3. Flowchart of information of the SSTI surveillance system

CHAPTER 2: Materials and Methods

NASAL COLONIZATION WITH *STAPHYLOCOCCUS AUREUS*

General Objective

The main objective of this study was to determine the natural history of nasal colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) among military personnel in a developing country (Peru). To meet this general objective we accomplished the following specific objectives:

1. To determine the risk factors associated with the nasal colonization with *Staphylococcus aureus*.

2. To assess the prevalence rates of baseline nasal colonization with methicillin-resistant *Staphylococcus aureus* in the military population of the Peruvian Air Force.

3. To assess change and the risk factors associated with change in the nasal colonization status with *Staphylococcus aureus* after 6 months of follow-up.

4. To determine the molecular profile and genotype of *S. aureus* isolates colonizing military personnel in four regions of Peru.

Study Design

We conducted a prospective cohort study with one year of follow-up among active duty military personnel from four bases of the Peruvian Air Force (Lima, Arequipa, Talara, and Iquitos). See Figure 4.

Study Population

The study population was comprised of male and female military active duty personnel, age 18 years and older, from the following base locations of the Peruvian Air Force (Lima, Arequipa, Talara, and Iquitos).

Figure 4. Map of Peru with the 4 study sites for the nasal colonization component.

The inclusion criteria for this study included:

- Active duty personnel of the Peruvian Air Force
- Personnel with less than two years of residence and who would be staying at least for one year at each base location
- Agreement to participate and signing of the informed consent

Sample size

For specific objectives 1 and 2 we calculated the sample size assuming that the proportions of baseline nasal colonization were similar to those reported by Ellis et al (23); the sample size required for obtaining a 95% confidence interval with a margin error of 5 percentage points for the three different groups of nasal colonization (MRSA colonized, MSSA colonized, and Non-colonized) was 450 participants. To allow us to model by location and other factors, we planned to include 250 participants per each region (4 in total), which gave a total of 1000 participants for this study.

In the case of specific objective 3 (change in the follow-up colonization rate), we defined our outcome as Non-colonized and Colonized. If the proportions of nasal colonization (MRSA and MSSA) at baseline are similar to those found by Ellis et al., and the correlations between the members of the pairs is 0.45 (calculated based on the results of Ellis et al. (24)) with 1000 participants we should expect a power above 80% for detecting a difference of 5 percentage points between the baseline colonization rates and the follow-up colonization rates with an alpha=0.05. We used the McNemar's test for paired proportions to calculate the power.

For specific objective 1 (assessment of risk factors for baseline nasal colonization), assuming that roughly 1/4 of the sample is positive for previous use of

antibiotics, previous hospitalizations and previous skin infections, and that the rate of baseline colonization with *Staphylococcus aureus* is 0.42; 1000 participants were sufficient to detect an increase of 0.25 in the odds ratio of each variable, with a power above 80% and an alpha=0.05**.**

Study Sites

Participants enrolled in the study were stationed at four bases, one in each region of the Peruvian Air Force. Each base had more than 250 active duty military personnel at the moment of the enrollment; and it was selected by the Personnel Command of the Peruvian Air Force, which authorized the execution of this objective only in these 4 bases (Appendix 1 – A. Peruvian Air Force authorization, B. NAMRU-6 IRB approval, and C. USUHS IRB approval). These bases were located at four urban centers, two in areas with higher temperatures (Iquitos is in the jungle, and Talara which is located in the northern desert coast). The other two base locations were Lima, which is located on the central coast with mild to warm temperatures depending on the season, and Arequipa which is located in the southern highlands, with a dry and relatively cold climate; is also the only city located at high altitude.

Study Procedures

Enrollment

We enrolled military personnel from the four largest bases in each Air Force region: Las Palmas Air Force Base at Lima (Center), Air Group 11 at Talara (North), Wing 4 at Arequipa (South) and Air Group 42 at Iquitos (East). At each center, we identified and followed-up a cohort of active duty military personnel (officers, Non-Commissioned officers, and enlisted personnel) with less than two years of deployment in each base, who

were selected using a simple random strategy. The Department of Health of the Peruvian Air Force was in charge of the sampling process using a table of random sampling numbers that was applied to a list of eligible participants at each study site. From each base, we invited 275 eligible participants to attend a lecture about *S. aureus*, MRSA and SSTIs that informed the participants about the study and answered questions they may have about the study. An ombudsman not related to the study was available to guarantee that the information provided about the study was adequate and accurate. At the end of the lecture participants were asked if they wanted to participate in the study. Those who indicated interest in the study were recruited. The participants of this study did not receive any kind of compensation.

Informed Consent

After the study was explained, and the participants agreed to be part of the study, we took them to a private area in the presence of the ombudsman, where the PI gave the informed consent to the participant and answered any question he/she may have about it. To prevent any appearance of coercion, the lectures were given separately for each rank, avoiding the presence of superior officers or non-commissioned officers and given by the Principal Investigator (civilian). After we obtained the signed informed consent (Appendix 2 – Informed Consent), the participant was enrolled in the study and a questionnaire about demographic and risk factors was administered. At each base, baseline nasal swabs were taken for assessing nasal colonization by *S. aureus*, including MRSA.

Baseline Questionnaire and Sampling

After the participant signed the informed consent, a self-administered questionnaire about demographic and clinical background was given to each of them (Appendix 3 –

Enrollment form). After completion of the questionnaire, a baseline nasal swab was taken. One member of the study team took a sample from the vestibular area of each of the nares, using BD BBL CultureSwabs® (BD Diagnostic, Sparks MD). The swabs were sealed in a tube and used exclusively for each participant. A sealed swab was opened and then, the swab was put on the anterior nares and rotated clockwise three times per each nare. Then, it was put in the tube with the enrichment media, and closed. Immediately after, it was put in a container to keep it refrigerated. After we finished taking all the samples, they were stored in a refrigerator at 4°C until the time of the shipping to NAMRU-6 labs; usually the time for shipping the nasal swabs was between three to four days at Iquitos, Arequipa and Piura; while at Lima it took 1 to 2 days. See Figure 5.

Figure 5. Nasal swab procedure

All the questionnaires and samples had a unique code per participant that was printed on labels. The creation of labels followed these steps: We hoped to recruit 250 from each location. We also expected to take 4% duplicate (10 duplicated samples total per site; 2 samples obtained from every 25th participant) samples to assess reproducibility, and 5 "known quality control" samples for the between location and time period variability assessment in each batch for a total of 265 samples per location-time period. Label numbers were generated from 10001-11060 for the baseline time period and then randomly sorted in blocks of 265 numbers. This random assorted list was with 6 labels per number. The first 5 of the random list within each block of 265 were used for the 5 "known quality control" swabs. The remaining 260 numbers of the list were used in their random printed order for the study subjects at each location. In preparation for shipping, the samples were ordered following an ascending numeric order. This process was repeated for each location.

At baseline, a list with the participant's name, CIP (Identification code for the Peruvian Armed Forces) and the study code (which was the same for all the forms belonging to each participant) was created and kept by the Department of Health of the Peruvian Air Force. The PI did not have access to this information. All the study forms (signed informed consents, enrollment questionnaires and follow-up questionnaires) were stored at the Data Coordinating Center, at NAMRU-6.

Follow-up Sampling

During the follow-up period, we planned to take two more nasal swabs from each recruited participant and obtain a follow-up questionnaire at 6 months and 1 year (Appendix 4 – Follow-up form). Also, we planned additional samples for the reproducibility analysis following similar procedures as baseline sampling. The labels for

the follow-up questionnaires and samples followed the same procedure described above. For time period 2 (6 months), the same process will be followed with numbers 20001-21060) and time period 3 (1 year) with numbers 30001-31060.

Lab procedures

Labeled specimens were shipped in a Styrofoam box with ice packs to the Naval Medical Research Unit N° 6 (NAMRU-6) located at Lima, Peru. Specimens received by the microbiology lab were logged into the project database and labeled according to the lab's standard procedures. A link to the study identification number was maintained. The swabs inside the box included the samples form the participants, the duplicate samples form some of them and the quality control samples. They were randomly mixed within each set of samples and the lab personnel were not aware that we included duplicate samples. After they were received, nasal culture specimens were placed in 5 ml of tryptic soy broth (TSB) supplemented with 6.5% NaCl and incubated for 18-24 hours at 35^0C. After that time, a 75 microL aliquot was plated onto mannitol salt agar. Plates were incubated for up to 48 hours at 37^0C and inspected for yellow colonies characteristic of *S. aureus*. Possible *S. aureus* isolates were sub cultured onto tryptic soy agar with 5% sheep blood. Subsequent colonies underwent catalase and coagulase testing per Micro Lab Standard Operational Procedures (SOP). All confirmed *S. aureus* isolates collected from the nasal swabs underwent susceptibility testing using disk diffusion tests, and they were performed using the standards established by the Clinical and Laboratory Standards Institute (80). Therefore, with these lab processes we identified MRSA strains and the antimicrobial susceptibility. All these procedures were performed at the NAMRU-6's Bacteriology labs. Later, samples of the identified MRSA strains were shipped to Dr. Michael Ellis' research laboratory at the

Uniformed Services University (USU) for the pulsed field gel electrophoresis (PFGE) procedures.

We used PFGE to characterize the MRSA strains. Based on the PFGE results, we classified the *S. aureus* isolates into pulsed-field type (PFT) (46). Analysis was aided by the use of *S. aureus* control strains of known PFT obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (http://www.narsa.net). We determined the presence of purported virulence factors and significant resistance factors in colonizing and clinical isolates of *S. aureus* using polymerase chain reaction (PCR) for Panton-Valentine Leukocidin (PVL), arginine catabolic mobile element (ACME), staphylococcal chromosome cassete (SCCmec) type, presence of toxic shock syndrome toxin (TST), gene ileS-2 for resistant to mupirocin (mupA), and tolerance to chlorhexidine (qacA/B) using protocols with which we have experience(23).

Report of Results

Nasal swab results were reported to our Point of Contact personnel (POC) who is a member of the Directorate of Health of the Peruvian Air Force. As researchers, we recommended the results be given to all the participants, in order to provide standard medical care. The Peruvian Air Force delivered results to the health facilities of each study site, and later they were given to each study participant from each base.

Ethical aspects

The use of human subjects is necessary to systematically evaluate the natural history of nasal colonization and infection with methicillin-resistant *Staphylococcus aureus* in deployed military personnel. The risks and benefits, as outlined in the informed consent, were explained to each subject, and the enrollment process also included the presence of a

trained ombudsman. Taking samples can cause slight discomfort to the participants. Nasal swabbing is a minimally invasive procedure, with little discomfort for the participant. Thus, there is an extremely small possibility for an adverse reaction; and the identification of a strain colonizing the nares can have important implications for further medical care of the participants; in case they develop infections associated with nasal colonization with MRSA. In addition we scheduled an informational meeting at the end on the study period to let the subjects know what the study results were and what they mean. This study itself only analyzed information identified through the study ID number.

Data collection was completed by the PI (Joan Neyra) and a member of the Peruvian Air Force. Nasal swab collection was conducted by a team composed of the PI, and a member of the Peruvian Air Force, at each study site. Each participant was assigned a unique code that served as a unique identifier during the study. No personal identifiers were used. Each questionnaire, informed consent and nasal swabs had the same code per participant (as described before). Data was collected at baseline, at 6 months and after one year at the four locations indicated. All official protocol files and databases will are maintained at the Bacteriology Program of NAMRU-6, which is the Data Coordinating Center. Baseline and follow-up questionnaires and laboratory results were stored securely at the Data Coordinating Center.

Data Analysis

Primary Endpoints

Overall nasal colonization

Nasal colonization status is defined in two groups: Colonized or Non-colonized. The overall nasal colonization proportion is defined as if any of the recruited participants was positive for nasal colonization with *Staphylococcus aureus* at any time of sampling.

Baseline nasal colonization rate

Nasal colonization status is defined in two groups: Colonized or Non-colonized. The baseline nasal colonization proportion is defined as positive if the first sample of any of the recruited participants was positive for nasal colonization with *Staphylococcus aureus*; otherwise it was negative.

Change in nasal colonization

This variable was defined as any change (negative to positive or positive to negative) that occurred in the nasal colonization status after a second sample was taken from a participant recruited at baseline.

Statistical Procedures

Calculation of nasal colonization rates

Overall and baseline colonization status are described using individual data based on gender, rank, base of recruitment, age (as categorical groups), smoking status, previous hospitalizations in the last 12 months, previous deployments, use of antibiotics in the last 12 months, previous skin and soft tissue infections in the last 12 months, previous respiratory diseases and use of corticosteroids in the last 12 months; and is reported as numbers and proportions with two-sided 95% confidence intervals. We also reported the results of the antimicrobial susceptibility for those isolates positive to *Staphylococcus aureus*.

To assess if there is a difference in the proportion of colonization with *Staphylococcus aureus* (Colonized vs. Non-colonized) in military personnel after the follow-up period, the post follow-up prevalence of nasal colonization was calculated. Prevalence of colonization with *Staphylococcus aureus* by the end of the follow-up period is compared against the baseline prevalence rates among those colonized (MRSA and MSSA) and non-colonized. A McNemar's test was used to assess if there was a significant difference among these groups. A p value <= 0.05 was considered as significant. We also report the prevalence rate of nasal colonization with MRSA strains.

Nasal carriage status over time

We defined the nasal carriage status using the index carrier used by Eriksen et al., which is defined as the number of positive swabs divided by the total number of swabs among those participants (n=186) who provided 3 samples during the study period. We defined 4 categories of nasal carriers: No carriers (index: 0), Occasional carriers (index: 0.1 – 0.4), intermittent carriers (index: 0.5 – 0.8) and permanent carriers (index: 0.9 – 1.0) (25). We reported them as proportions.

Losses to follow-up

We divided the study population in two groups: those who were lost to follow-up and did not provide a second sample at any point of time, and those who provided a second sample (either at 6 months or 1 year). We compared the baseline demographic and clinical characteristics between both groups to assess if there was a statistically significant difference between them. In addition, we compared if there was a difference between the measures of association of the risk factors for baseline nasal colonization between those who were lost during the follow-up period and those who remained as study participants.

Assessment of risk factors associated with baseline nasal colonization

To determine the risk factors associated with baseline nasal colonization, a logistic regression model was used to determine the association of the following variables: age, sex, rank, region, time of service, smoking status, occupation, number of deployments during the last year, use of antibiotics in the last 12 months, previous hospitalizations in the last 12 months, skin and soft tissue infections in the last 12 months, and use of corticosteroids in the last 12 months. The logistic regression procedure used the following steps:

1. Before the model building, we performed a descriptive analyses of all the variables considered for modeling: assessing the distribution, presence of empty cells for categorical variables, assessing collinearity between some variables and evaluating the linearity assumption between the log-odds of the outcome and the continuous variables considered for modeling. In case these variables are not linear, we considered the transformation of some of them.
2. Our intention was to create a model including all of the four main independent variables: use of antibiotics in the last 12 months, use of corticosteroids in the last 12 months, previous hospitalizations in the last 12 months, previous skin and soft tissue infections in the last 12 months with the dependent variable of baseline colonization status (Colonized with *S. aureus* / Non-colonized with *S. aureus*); and we considered for the analysis of confounding the following variables: gender, rank, base of recruitment, age (as categorical groups), previous respiratory diseases, smoking status.

3. To define a confounding variable, we assessed the association between each confounding variable with the main outcome and one of the three main independent variables. Once it is established either an *a priori* association or a statistical significant association (p value < 0.20) between them in this study data, we considered each of the additional variables as a confounders and proceeded to include them into the logistic model.
4. The model building included the four main independent variables (Model 1). After this, we added one of the confounding variables, at each time and we compared them with the Model 1, using the likelihood ratio test. The confounding variable with the lowest p value obtained in the LR test was introduced in the model 1 and became the new model for comparison.
5. We repeated step 4 for each of the confounding variables until there were no more variables to include, or they did not add any significant statistical change into the overall model. However, the main criterion for including a variable and confounders in the model was the epidemiological criteria.
6. Later, after we determined the final model, we performed an overall goodness of fit test.

Assessment of risk factors associated with change in nasal colonization

To determine the risk factors associated with change of nasal colonization status with *Staphylococcus aureus*, a multinomial logistic regression was used to determine the association of this outcome (no change, positive to negative, negative to positive) with the following variables: use of antibiotics, use of corticosteroids, and diagnosis of skin and soft tissue infections during the follow-up period. These variables were evaluated for

confounding: sex, region of recruitment, and deployments during the last year. The procedures described previously were used for building the logistic regression model.

Reproducibility analysis

For the assessment of quality of our lab procedures, we performed two small sub-studies. First, to evaluate the variability of the lab tests for identifying *Staphylococcus aureus* at each location (Lima, Chiclayo, Arequipa, and Iquitos) and sampling time (Baseline, 6 months and 1 year) we took a second sample from some participants (we planned 120 repeated samples). Therefore, we had two samples taken from the same participant at the same location and time. The labeling of these duplicate samples followed the exact same procedure to those put on the other nasal swabs.

We calculated the overall positive percent agreement (PPA) in these duplicate samples, comparing the results of the culture of the first sample with the second sample taken form the same person (if they were *Staphylococcus aureus* positive or negative). In addition to the overall PPA, we also calculated the PPA by time of sampling (baseline, 6 months, and 1 year), by operator (person in charge of taking the nasal swab, and by place of recruitment (Iquitos, Arequipa, Talara and Lima). All repeated samples were shipped as part of the total swabs sent to NAMRU-6's lab. There, they underwent the lab procedures described previously to identify *Staphylococcus aureus*. NAMRU-6 sent a report of the results and we built a specific dataset for the reproducibility analysis, comparing the results of the first sample with the second one; and therefore, we could calculate the PPA.

Second, to evaluate the impact of shipping variations between time periods and locations, we selected a sample with *Staphylococcus aureus* from the research lab and we "created" nasal swabs from this sample at each sampling time (Baseline, 6 months and 1

year) so that they should all be identical. These samples were labeled following the same procedures described for the other nasal swabs in the study. For the “created” samples that should be "identical" we evaluated whether there was a location or time effect as well as "team-member" effect using a regression to see if there is any of these factors significantly influenced the determination of *Staphylococcus aureus* status.

At each location, after the nasal swabbing was finished, all the regular samples plus the repeated samples and “created” samples were put in a box (or boxes) for shipping to the NAMRU-6 labs. The samples that had been labeled in random order were put in numeric order for shipping. The shipment included the repeated nasal swabs and the “created” nasal swabs from the research team member (mixed with the regular samples, prepared before the beginning of the sampling and taken to the location). Therefore, the lab technicians were unaware of the existence of the repeated and “created” samples.

SKIN AND SOFT TISSUE INFECTION SURVEILLANCE

General Objective

As part of this study we developed an epidemiological surveillance process for SSTIs in the Peruvian Air Force through the implementation of reporting of individual SSTI events in the current electronic disease surveillance system. Our specific objectives were:

1. To determine the prevalence rates of reported skin and soft tissue infections (SSTIs) in the Peruvian Air Force during the period 2011-2014.

2. To include the reporting of skin and soft tissue infections in the Peruvian Air Force’s electronic surveillance system of infectious diseases.

3. To evaluate the surveillance of skin and soft tissue infections after 9 months of implementation.

Study Design

We implemented the surveillance of skin and soft tissue infections in the current electronic epidemiologic surveillance system of the Peruvian Air Force at 27 health facilities of the Peruvian Air Force, which allowed us to evaluate surveillance data from the entire active military population (approximately 9000 active military personnel in the Peruvian Air Force). After 9 months, we evaluated the performance of the SSTI surveillance system implemented in Peruvian Air Force.

Study Population

We included the total Peruvian Air Force active military population (officers [commissioned officers], Non-Commissioned officers [warrant and petty officers], cadets [commissioned officers in training], alumni [non-commissioned officers in training], and troops (sergeants, corporals and privates) during the period 2011-2014. During this period, the electronic surveillance system covered 100% of all units in the Peruvian Air Force; and these units were fully incorporated into the system after the training period.

Procedures

We added the report of skin and soft tissue infections to the current functioning electronic surveillance system of infectious diseases implemented in the Peruvian Air Force on April 2014. Our case definition was based on the depth of the lesion. We defined SSTIs as all the clinical events that affect the dermis, epidermis, superficial fascia, and

subcutaneous tissue. These clinical entities include: erysipelas, impetigo and folliculitis (epidermis); ecthyma, furunculosis, and carbunculosis (dermis); cellulitis (superficial fascia); and necrotizing fasciitis (subcutaneous tissue).

For reporting the events, we modified a previously validated questionnaire from the electronic surveillance system already functioning in the Peruvian Air Force. It included demographic data, and clinical information about each SSTI diagnosis (Appendix 5 – SSTI Report Form). After the questionnaire was designed, it was included in the system as an "Immediate Report" (a report is filled out and entered into the system as soon as the case of SSTI is diagnosed at the health facility) and tested for performance by the Bioinformatics Unit at NAMRU-6. After the test was successful it was delivered for use. See Figure 4. The training of the stakeholders included two phases: First, an on-site visit to all the reporting bases of the Peruvian Air Force; and second, continuous refresher sessions online or by phone were scheduled every quarter in charge of the Peruvian Air Force's system administrator.

The on-site training was focused on collecting the information from the log-books, completing the questionnaire, sending the report by Internet. We provided training materials for them, which included epidemiologic calendar, definition of SSTIs, and how to report into the electronic system. (See Appendix 6 – Training material). After the training session, each stakeholder was able to enter the report into the surveillance system through the Internet (See Figure 6). At each health facility, they had to identify the SSTI cases, identifying the following variables for each case: age, gender, rank, date of diagnosis, diagnosis, ICD-10 code and treatment (Appendix 7 – Log-Book collection form).

Along with the training of the stakeholders, we reviewed the log-books at each health facility, looking for all the SSTI cases reported during the period 2011 – 2014, in order to calculate the prevalence rates for each year. When log-books were absent, we reviewed the prescriptions or obtained the de-identified dataset with all the reported cases. The diagnosis included were Impetigo, Abscess, Furunculosis, Folliculitis, Cellulitis, lymphadenitis, Pyoderma, and Infected wound, excluding surgical wounds. In the case that the diagnosis was not included, but there were ICD-10 codes, we included the following: L01. L02. L03, L04, and L08. For the calculation of the denominators, the Peruvian Air Force provided the approximate number of active military personnel at each military base where there was a health facility. The overall population was provided and also stratified only by gender and rank. Total numbers by each region and military base were not available due to national security issues.

Figure 6. Internet report form

Data Analysis

Primary Endpoints

The primary endpoints for our analyses of the disease surveillance system for SSTI reporting included:

- Determination of the prevalence rate of skin and soft tissue infections in the active duty military personnel of the Peruvian Air Force during the period 2011-2014
- Identification of the number of cases reported per month in the surveillance system
- Identification of the number of cases per site per week in the surveillance system

Statistical Procedures

SSTI prevalence

The prevalence of SSTIs was calculated using the prevalence over the one-year period. The information was obtained from the log-books of each health facility of the Peruvian Air Force at the beginning of the study. For the calculation of the rates within gender and rank categories, the approximate number of service personnel at risk within each category during the time interval was provided by the Department of Health of the Peruvian Air Force. SSTIs are described as individual data based on gender, rank, location, time of infection, diagnosis and whether they received antibiotic treatment; and will be reported as numbers and proportions with two-sided 95% confidence intervals. We compared the differences in the rates of SSTIs based on gender and rank. Tests of significance were evaluated using the Z-statistic and p values were reported.

Evaluation of the SSTI surveillance system

We monitored the performance of the SSTI surveillance system during the follow-up period (9 months). It included the assessment of the following aspects:

1. Usefulness:
    - Number of cases reported per month
    - Number of cases per site per week
2. Attributes:

- Data quality was calculated using (1) the completion of reports proportion (number of fully completed reports/total number of reports); and (2) the error per report proportion (number of errors/total number of reports). Both indicators were calculated per month. We expected a proportion lower than 5% for both indicators to define good data quality.
- Sensitivity of the system was calculated at the end of the follow-up period (9 months) and was referred to the sensitivity of case reporting. We used as a gold standard the cases reported in the log-books of each of the 27 Health facilities of the Peruvian Air Force, looking for the cases reported in the system during the follow-up period. Sensitivity of case reporting was defined as number of cases detected by the SSTI surveillance system divided by the total number of cases recorded in the log-books. We expected to reach more than 80% of sensitivity of the case reporting (39; 79). We also compared the incidence of SSTIs reported at the four bases where the education program and screening program was ongoing to that of other Peruvian Air Forced bases.

- Timeliness was calculated as the timeliness of receipt of surveillance reports defined as the number of on-time reports/total number of reports. This indicator was calculated per month. We expected a proportion above 80% (79).
- Stability of the system was calculated as (1) the number of system failures per time of functioning of the SSTI surveillance system and (2) the duration of downtimes (28).

Report of Results

The results were delivered to the Directorate of Health of the Peruvian Air Force, including our prevalence rates of SSTIs, their distribution by year, age, gender, region and rank. These results and those from the nasal colonization study are summarized in Appendix 8 (Executive Summary in Spanish).

CHAPTER 3: Results

OBJECTIVE 1: NASAL COLONIZATION WITH *STAPHYLOCOCCUS AUREUS*

Study Population

The study started in October 2013, and three visits were scheduled at each study site (Iquitos, Arequipa, Talara and Lima) during the following periods: October-November 2013 (baseline visit), April – August 2014 (6 months visit) and October – December 2014 (1 year visit). The baseline visit was intended to recruit participants at each site (1700 potential participants) and reach the required 1000 participants estimated by the sample size calculation. (250 per each study site), while the 6 month and 1 year visit were scheduled to take the follow-up samples from the recruited participants.

During the baseline visit, we were able to recruit 655 participants at the four study sites. In order to increase our sample size number, we enrolled 101 additional participants during the 6 month visit (April – August 2014). No more participants were recruited at the 1 year visit. Therefore we ended up with 756 recruited participants. Of the original 655 participants enrolled at the baseline visit, we were able to obtain a follow-up sample (at 6 months) from 327 participants, having lost almost 50% of the initial recruited subjects. See Figure 7 for details regarding loss-to-follow-up.

At the 1 year visit, we collected follow-up samples from 343 participants; 280 of those recruited at the baseline visit, and 63 of the 101 participants recruited during the 6 month visit. Of the 280 participants recruited at the baseline visit who were sampled at the 1 year visit (November – December 2014), 186 provided a third sample having completed the all follow-up encounters; while 94 provided a second sample one year after the initial

sample was taken. Therefore, only 186 participants completed all the activities initially planned in the study. See Figure 8 for the number of participants sampled at each visit.

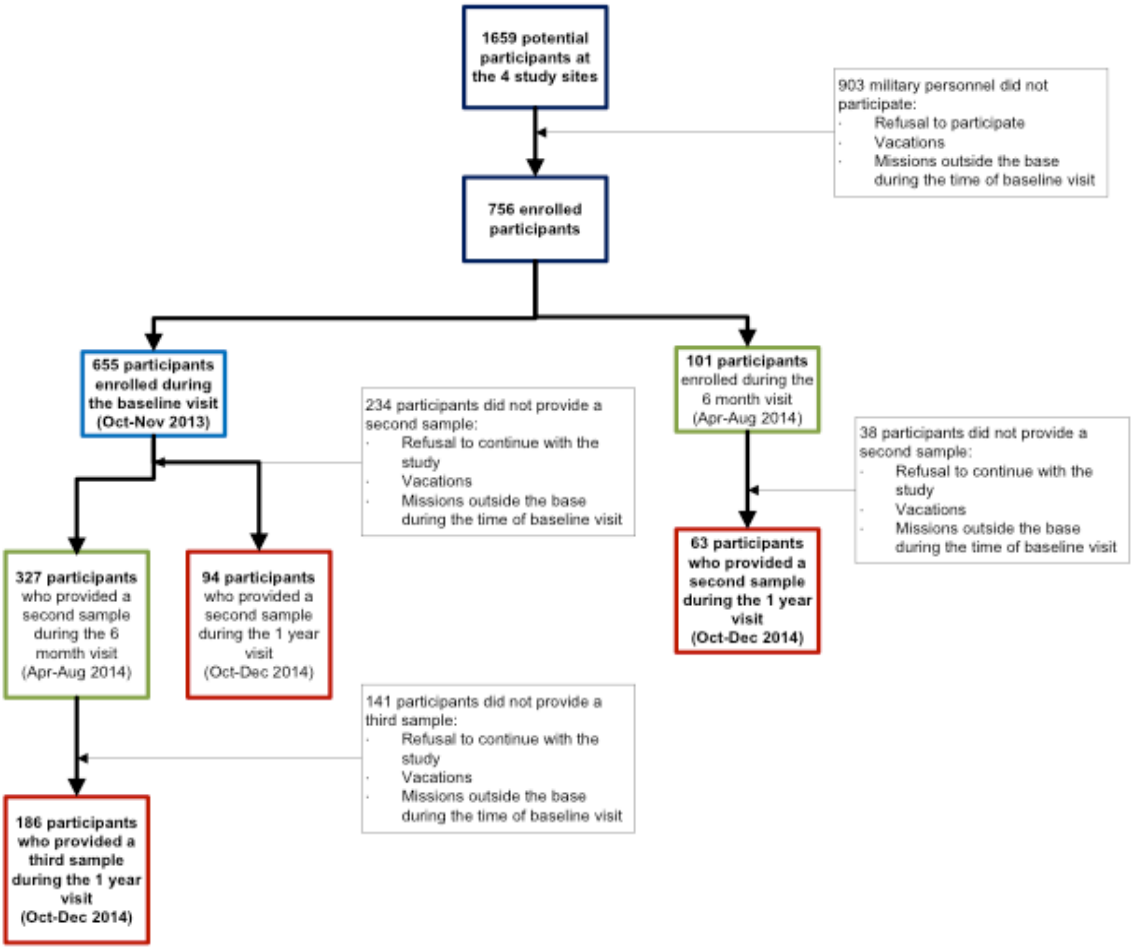


Figure 7. Number of study participants at each visit
In blue are highlighted those participants who were sampled at the baseline visit (October – November 2013). In green are those who provided a sample at the 6 month visit (April – August 2014) and in red are those who provided a sample at the 1 year visit (October – December 2014).

The main reasons for the loss to follow-up of the 272 participants were discharge or retirement (n = 99, 36.4%); deployment to other bases (n = 75, 27.6%); temporal

unavailability at the time of the second or third visit due to leaves, commissions outside the base, and vacations (n = 38, 13.9%); and death (n = 1, 0.4%). Of the total number of participants, 756 who provided at least 1 nasal swab, 390 participants provided at least two samples with 6 months between, 94 participants provided at least two samples with a 1 year interval, and 186 participants provided 3 samples (baseline, 6 months and 1 year). Therefore, our overall loss to follow-up rate by the time of the end of the study was 71.6% (186 of 655). However, we were able to obtain a second sample from 484 participants at some point of time during the follow-up period (6 months to 1 year), and then our loss to follow up at this point was 35.9%. Table 2 shows the distribution of samples by each study site.

Table 2. Distribution of samples by study site

Study Site	Potential participants at each study site	Number of participants enrolled in Oct-Nov 2013	Number of participants enrolled in Apr-Aug 2014	Total number of participants with one sample (%)	Total number of participants with two samples (%)	Total number of participants with three samples (%)
Iquitos	407	191	63	254 (62.0)	163 (64.0)	68 (27.0)
Arequipa	300	130	34	164 (55.0)	110 (67.0)	44 (27.0)
Talara	345	157	4	161 (47.0)	114 (71.0)	44 (27.0)
Lima	607	177	0	177 (29.0)	97 (55.0)	30 (17.0)
Total	1659	655	101	756 (46.0)	484 (64.0)	186 (25.0)

Demographic characteristics

The Peruvian Air Force (PAF) has approximately 10000 active duty military personnel, distributed in four wings (with the largest being administrative and operational divisions), one in each of these regions. The PAF personnel are deployed to each of these Wings located in Iquitos (east), Arequipa (south), Chiclayo (north), and Lima (center). We were authorized to conduct the study in 3 of these cities, but in the north we were restricted

to Talara (GRU11), which is a combat unit. Usually each active duty personnel is deployed to these units for two years, starting during the first week of January for the officers and Non-Commissioned officers. The troops are the enlisted personnel at each base, and usually they serve only for two years. The deployment of personnel does not imply that further deployments cannot occur. One example is the deployment to the VRAE region (Apurimac and Ene Rivers Valley), an area with active military operations. Also, the personnel can ask for discharge from duty at any point of time, which is more frequent in the troops.

The mean age of the study participants was 30.3 ± 11.5 years. We decided to group age into four categories (18 – 29 years, 30 -39 years, 40 -49 years and 50 years or older). Almost 60% of the study population was younger than 30 years. The mean time of service at the time of enrollment was 10.5 ± 10.9 years. We categorized the time of service in three categories (10 or less years of service, 11 to 20 years, and 21 or more years), and 61% of the participants had up to 10 years of service in the Peruvian Air Force. In terms of gender, 80.7% were male and 19.3% were female; these proportions were similar to those found in the total active duty military personnel of the Peruvian Air Force (17.5% female vs. 82.5% male).

We grouped the ranks in three categories: Officers (Generals, Colonels, Commanders, Captains, and Lieutenants), Non-Commissioned Officers (Technicians, and Sub-Officers) and Troops (Sergeants, Corporals and Airmen). In our study population, officers were only 11.1%; and Non-Commissioned Officers and troops had similar proportions (48.7% and 40.2%, respectively). These proportions differ from those of the active duty military personnel of the Peruvian Air Force, where 15.4% are officers, 57.1% the Non-Commissioned officers and 20.2% troops. In our study population, the troops were

over-represented. Of the 756 recruited participants, Iquitos provided 33.5% while Arequipa and Talara provided 21% each and Lima 23.4%.

Table 3. Demographic characteristics of study participants

Variable	Frequency (756)	Percentage (%)
Age		
18 – 29 years	450	59.5
30 – 39 years	95	12.6
40 – 49 years	151	20.0
50 years or more	60	7.9
Time of service		
10 years or less	463	61.2
11 – 20 years	103	13.6
20 years or more	190	25.1
Sex		
Female	146	19.3
Male	610	80.7
Rank		
Officers	84	11.1
Non-Commissioned Officers	368	48.7
Troops	304	40.2
Base of recruitment		
Iquitos	253	33.5
Arequipa	164	21.7
Talara	162	21.4
Lima	177	23.4
Administrative activities		
No	510	67.5
Yes	246	32.5
Instruction activities		
No	483	63.9
Yes	273	36.1
Combat activities		
No	535	70.8
Yes	221	29.2
Number of activities		
Not related	106	14.0
Unique	580	76.7
Multiple	70	9.3

Place of residence		
Barracks	250	33.1
Inside the base	114	15.1
Outside the base	392	51.9

The occupations of the study subjects included administrative (32.5%), instructional (36.5%) and combat (29.2%) activities. 76.7% performed only one of these activities, 9.3% reported as having more than one activity; and 14% indicated that none of these three activities described their occupation. Administrative tasks included office work, meetings, and coordination activities between the different departments at each base. Instruction activities involved the training of the troops, which is performed by non-commissioned officers, sergeants and corporals. They included not only theoretical instruction but also physical training performed by the trainers and trainees (this training not only included physical activity but also combat rehearsals). Combat activities involved combat operations and rehearsals as part of the military readiness. Regarding the place of residence, more than half of the study population lived outside the bases (51.9%), while a third lived in the barracks (33.3%). See Table 3 for the distribution of the demographic characteristics of the study population.

Clinical characteristics

Medical conditions

We asked each participant for previous medical conditions, using the diseases listed in the Enrollment Questionnaire form. In our analysis we included all diseases, which were likely treated with antibiotics, including gastrointestinal and liver diseases, respiratory diseases (asthma, chronic obstructive pulmonary disease and chronic bronchitis), heart conditions (heart disease, hypertension) infectious diseases (among them tuberculosis,

malaria, typhoid fever and dengue), diabetes, kidney diseases, and skin diseases. 152 participants (20.1%) reported having a medical condition; of them, 118 (15.6%) only reported one disease, while 34 (4.5%) reported having more than one disease. Almost 80% of the study population did not report any medical condition. When we analyzed the specific categories of diseases, 10% reported gastrointestinal diseases, 5.4% reported a skin disease, 2.6% reported a respiratory disease, and 1.1% reported an infectious disease. See Table 4 for the clinical characteristics of the participants.

Table 4. Clinical characteristics of the participants.

Variable	**Frequency**	**Percentage (%)**
Medical conditions		
No	604	79.9
Yes	152	20.1
Number of medical conditions		
None	604	79.9
One disease	118	15.6
More than one disease	34	4.5
Use of antibiotics in the previous year		
No	427	56.5
Yes	263	34.8
Unknown	66	8.7
Use of corticosteroids in the previous year		
No	536	70.9
Yes	153	20.2
Unknown	67	8.9
Hospitalized during the previous year		
No	645	85.3
Yes	88	11.6
Unknown	23	3.0
Diagnosis of SSTIs during the previous year		
No	671	88.8
Yes	44	5.8
Unknown	41	5.4
Smoking status		
Never	345	45.6

Past smoker	126	16.7
Current smoker	259	34.2

Use of antibiotics

When asked about the use of any antibiotic during the previous year, 34.8% of the participants reported the use of these medications, while 56.5% did not use them; and 8.7% did not know or remember if they had taken them. Of those who answered affirmatively (263), only 136 gave a specific antibiotic. After analyzing the entire group of participants, penicillins were the most used antibiotics (9.8%), followed by fluoroquinolones (4.2%), macrolides (2.9%), cephalosporins (0.9%), lincosamides and sulfonamides (0.8% each), and metronidazole (0.5%). If we looked at the penicillins group, the most used were amoxicillin (6.6%) of the total antibiotic user group and dicloxacillin (1.6%).

There is a separate Department of Pharmacy (SESAN) in charge of the acquisition, distribution, and surveillance of the medication (including antibiotics and corticosteroids) used by the Peruvian Air Force. Unfortunately this unit collects all copies of the prescription receipts and entered them into a dataset. Unfortunately, the Department of Health of the Peruvian Air Force does not have free access to this information; and therefore we could not match our findings with those stored in the mentioned datasets.

Use of corticosteroids

We asked the participants about the use of any corticosteroid in the previous year. 20.2% reported the use of this medication, while 70.9% did not use it; and 8.9% of the participants reported that they did not know or did not remember. When asked specifically if they remembered the corticosteroid that they used, only 29 of the 153 participants who reported having used this medication gave an answer. Of them, 23 had used dexamethasone

(which corresponds to 3% of all the enrolled participants, while less than 1% has used prednisone and clobetasol propionate cream As happened with the use of antibiotics, we could not match our findings with those stored in the datasets of the Department of Pharmacy of the Peruvian Air Force.

Previous hospitalizations and diagnosis of SSTIs

11.6% of the enrolled participants were hospitalized during the previous year, 85.3% reported no hospitalization and 3% did not answer the question. We did not ask the specific reason for hospitalization nor the duration of hospitalization. Regarding the diagnosis of any skin and soft tissue infection during the previous year, only 5.8% reported a SSTI, while almost 89% reported no diagnosis with these conditions. Only 5.4% did not remember or did not know. We did not ask about the specific SSTI.

Smoking status

We asked if the participant had smoked at any time of his/her life, if they had smoked during the last year; and the approximate number of cigarettes they had smoked during this last year. With these three questions we built a variable named "Smoking status" with 4 categories: never smoked, past smokers, current smokers. 45.6% never smoked during their lifetimes, 16.7% are past smokers and 34.2% are current smokers.

Nasal colonization prevalence

Our overall prevalence of nasal colonization (total number of participants with at least one sample positive for *Staphylococcus aureus* [143] / total number of participants) [756]) was 18.9% (95% CI: 16.1% - 21.7%) during the period of study. Our overall rate of

colonization with MRSA was 0.3% during the study period (2 of 756). Two isolates were MRSA and were both collected during the 6 month visit, one in a new enrollee (recruited in the study in May 2014), and the other from a participant who was enrolled in October 2013).

Baseline nasal colonization with Staphylococcus aureus

The baseline nasal colonization prevalence among the 756 participants enrolled in the study was 9.7% (n=73, 95% CI: 7.6 – 16.9). There were two periods of recruitment, in October-November 2013 (655 participants) and April-August 2014 (101 participants), and the baseline nasal colonization rates for each period were similar (9.8% vs. 8.9%, p=0.7853, Z-statistic for comparison of two proportions). We evaluated demographic and clinical variables to determine if there was a statistically significant association between prevalence of nasal colonization at baseline and these variables. The results are in Table 5.

Table 5. Prevalence of baseline nasal colonization among the different variables under study (N = 756, 73 positive and 683 negative)

	Baseline Nasal colonization (%, 95% CI)		
Variable	**Positive (n = 73)**	**Negative (n = 683)**	**P value**
Age			
18 – 29 years	10.2 (7.4 – 13.0)	89.8 (86.9 – 92.6)	0.537[a]
30 – 39 years	6.3 (1.4 – 11.2)	93.7 (88.8 – 98.6)	
40 – 49 years	11.3 (6.2 – 16.3)	88.7 (83.7 – 93.8)	
50 years or more	6.7 (0.3 – 13.0)	93.3 (86.9 – 99.7)	
Time of service			
10 years or less	10.6 (7.8 – 13.4)	89.4 (86.6 – 92.2)	0.334
11 – 20 years	5.8 (1.3 – 10.4)	94.2 (89.6 – 98.7)	
20 years or more	9.5 (5.3 – 13.7)	90.5 (86.3 – 94.7)	
Sex			
Female	6.8 (2.7 – 10.9)	93.2 (89.0 – 97.3)	0.201
Male	10.3 (7.9 – 12.7)	89.7 (87.3 – 92.1)	

Rank			
Officers	9.5 (3.2 – 15.8)	90.5 (84.2 – 96.8)	0.340
Non-Commissioned Officers	8.2 (5.3 – 10.9)	91.8 (89.0 – 94.7)	
Troops	11.5 (7.9 – 15.1)	88.5 (84.9 – 92.1)	
Base of recruitment			
Iquitos	9.1 (5.5 – 12.6)	90.9 (87.4 – 94.5)	0.023
Arequipa	14.0 (8.7 – 19.4)	85.9 (80.6 – 91.3)	
Talara	4.3 (11.8 – 7.5)	95.7 (92.5 – 98.8)	
Lima	11.3 (6.6 – 15.9)	88.7 (84.0 – 93.4)	
Number of activities			
Not related	10.4 (4.5 – 16.2)	89.6 (83.8 – 95.5)	0.587
Unique	9.1 (6.8 – 11.5)	90.9 (88.5 – 93.2)	
Multiple	12.9 (4.9 – 20.8)	87.1 (79.2 – 95.1)	
Number of medical conditions			
None	9.9 (7.5 – 12.3)	90.1 (87.7 – 92.5)	0.122
One disease	6.1 (1.7 – 10.5)	93.9 (89.5 – 98.3)	
More than one disease	17.6 (4.6 – 30.7)	82.4 (69.3 – 95.4)	
Respiratory diseases the previous year			
No	9.1 (7.0 – 11.2)	90.9 (88.8 – 92.9)	0.002
Yes	30.0 (9.4 – 50.6)	70.0 (49.4 – 90.6)	
Use of antibiotics the previous year			
No	9.1 (6.4 – 11.9)	90.9 (88.1 – 93.6)	0.598
Yes	11.0 (7.2 – 14.8)	88.9 (85.2 – 92.8)	
Unknown	7.6 (11.3 – 14.0)	92.4 (85.9 – 98.9)	
Use of dicloxacillin previous year			
No	9.3 (7.2 – 11.4)	90.7 (88.6 – 92.8)	0.005
Yes	33.3 (5.4 – 61.2)	66.7 (38.8 – 94.6)	
Use of corticosteroids the previous year			
No	8.9 (6.5 – 11.4)	91.0 (88.6 – 93.5)	0.432
Yes	12.4 (7.2 – 17.7)	87.6 (82.3 – 92.8)	
Unknown	8.9 (2.1 – 15.9)	91.0 (84.1 – 97.9)	
Hospitalized during the previous year			
No	9.6 (7.3 – 11.9)	90.4 (88.1 – 92.7)	0.971
Yes	10.2 (3.8 – 16.6)	89.8 (83.4 – 96.2)	
Unknown	8.7 (0.0 – 20.5)	91.2 (79.5 – 100)	
Diagnosis of SSTIs			

during the previous year			
No	9.5 (7.3 – 11.8)	90.5 (88.2 – 92.7)	0.848
Yes	9.1 (0.5 – 17.7)	90.9 (82.3 – 99.5)	
Unknown	12.2 (5.2 – 22.4)	87.8 (77.6 – 97.9)	
Smoking status			
Never	8.1 (5.2 – 11.0)	91.9 (88.9 – 94.8)	0.297
Past smoker	12.7 (6.9 – 18.5)	87.3 (81.5 – 93.1)	
Current smoker	10.4 (6.7 – 14.2)	89.6 (85.8 – 93.3)	
Place of residence			
Barracks	11.2 (7.3 – 15.1)	88.8 (84.9 – 92.7)	0.198
Inside the base	5.3 (1.1. – 9.4)	94.7 (90.6 – 98.9)	
Outside the base	9.9 (6.9 – 12.9)	90.1 (87.1 – 93.0)	

[a] We used the Fisher's exact test. For the rest of the variables, we used the Pearson chi square test.

The age groups with a higher prevalence of baseline nasal colonization with *Staphylococcus aureus* were those 18 to 29 years old and 40 to 49 years old (10.2% vs. 11.3%); in the other two groups, the prevalence was lower than 7%. Those with less than 10 years of service had a prevalence of 10.6%, similar to those with 20 to more years of service (9.5%). In terms of distribution by gender, there was a difference of 4 percentage points between male and female (10.3 vs. 6.8%, p=0.201). The troops had 11.5% of nasal baseline nasal colonization, higher than officers or non-commissioned officers (9.5% vs. 8.2%). There was a statistically significant difference in the distribution of nasal colonization by base of recruitment (p=0.023); Talara had the lowest baseline prevalence (4.3%) compared to the other bases that had similar rates (9.1% for Iquitos, 14.0% for Arequipa and 11.3% for Lima). The number of job activities performed by the participants did not show any statistical difference in the prevalence of nasal colonization. Regarding the place of residence, those who lived on base but not in the barracks had the lowest prevalence (5.3%) but this was not significantly (p= 0.198) different from those who lived at the barracks (11.2) and those who lived off the base (9.9%).

In terms of medical conditions, those who reported more than one disease had a prevalence of 17.6%, which was higher but not significantly different from those without disease and those who reported only one condition (9.9% vs. 6.1%). Those with respiratory diseases (asthma, chronic obstructive pulmonary disease and chronic bronchitis) had a statistical significant difference (p= 0.002) in the prevalence of nasal colonization (30%) than with those without a reported respiratory disease (9.1%). Smoking did not affect the prevalence of nasal colonization; the rates were similar among those who never smoked (8.1%), those who are previous smokers (12.7%) and those who currently smoke (10.4%) (p= 0.297).

Among those who reported the use of antibiotics during the last year, the nasal colonization prevalence at baseline was 11.0%, which was similar to those who did not use antibiotics (9.1%). However, when we analyzed the use of dicloxacillin the previous year (one of the most used antibiotics in Peru for any superficial skin and soft tissue infection), those who reported its use had a 33.3% prevalence of nasal colonization, compared with those who did not report its use (9.3%, p=0.005). Regarding the use of corticosteroids, those who reported use during the previous year, had a prevalence of 12.4%, which was not significantly different from to those who did not use it (8.9%, (p=: 0.432).

In terms of hospitalization during the previous year, the rates were similar among those who were hospitalized (10.2%) and those who were not (9.6%) (p=0.971). Similarly there was no difference in prevalence between those who had the diagnosis of skin and soft tissue infections during the last year (9.1%) and those who did not (9.5%).

Nasal colonization by time of sampling

When we analyzed the prevalence at each time of sampling, we found that the prevalence of nasal colonization increased with time of study. We started in October-November 2013 (baseline visit) with a baseline nasal colonization of 9.8% in a total population of 655 participants. At the second visit, which corresponded to the 6 month period of follow-up for 327 and the baseline for 101, our rate of nasal colonization was 12.4% among the 428 participants who provided a sample at this time. During our third study visit on October-December 2014 (1-year follow-up for some and 6 month follow-up for others), the rate was more than 2 fold higher compared with the baseline rate; with 20.4% prevalence. Figure 8 shows the significant difference between the prevalence at the third visit and the other two.

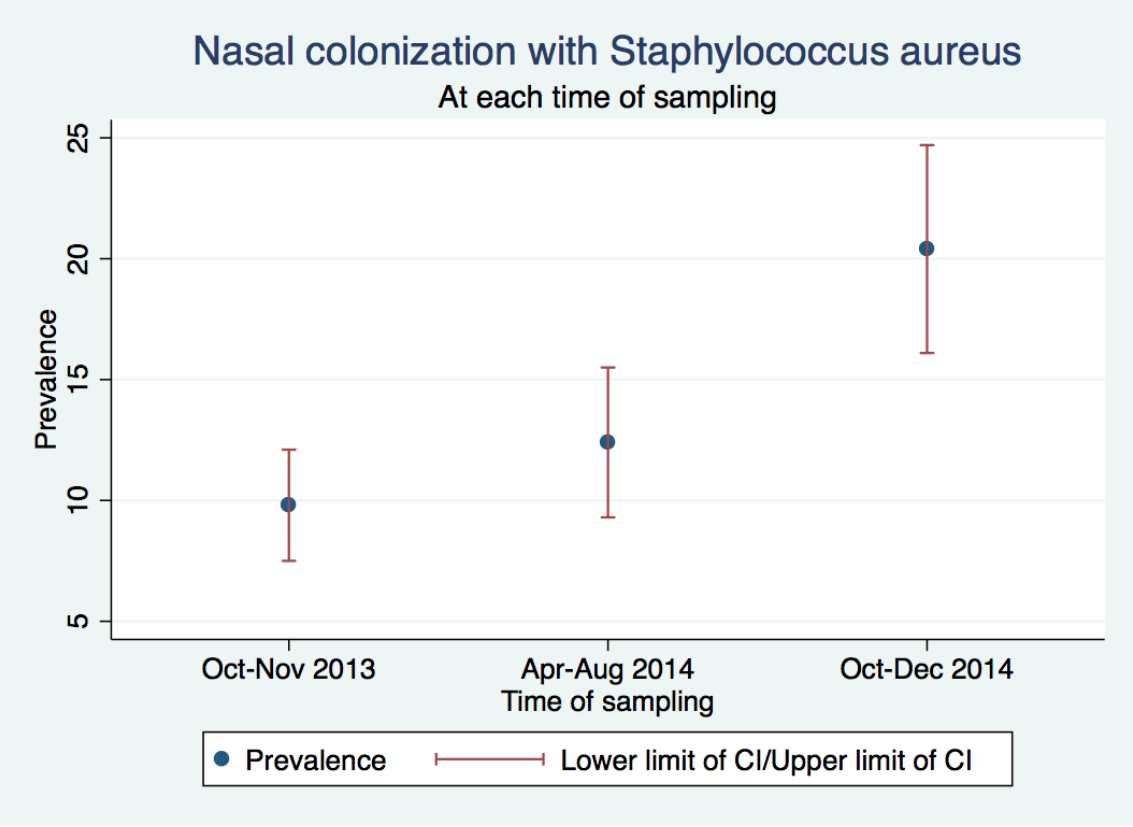


Figure 8. Prevalence of nasal colonization at each time of sampling

Antimicrobial susceptibility of positive isolates and MRSA isolates

NAMRU-6 and USUHS labs processed 183 positive samples from the participants during the study period. These samples showed a remarkable antimicrobial susceptibility (See Table 6). All of them were susceptible to ceftaroline, trimethoprim – sulfamethoxazole, vancomycin, and linezolid. The higher resistance was to erythromycin (16.4%), followed by gentamicin (3.3%). In addition, 6.6% had an inducible resistance to clindamycin.

Two isolates (1.1%) were methicillin-resistant *Staphylococcus aureus*. These two samples were collected in Arequipa. These two MRSA isolates were PFT USA100, SCCmec type II, close to the New York/Japan strain which has been reported to be circulating in Latin America. They also exhibit resistance to chlorhexidine, do not possess the Panton-Valentine Leukocidin, (PVL), nor the gene encoding the toxic shock syndrome toxin (TST). They possess genes that encode recombinases (ccrB2), but are susceptible to mupirocin.

Table 6. Antimicrobial susceptibility of 183 *Staphylococcus aureus* isolates

	Number (%) of samples		
Antibiotic	**Susceptible**	**Resistant**	**Intermediate**
Clindamycin [a]	179 (97.8)	4 (2.2)	-
Erythromycin	153 (83.6)	30 (16.4)	-
Doxycycline	180 (98.4)	-	3 (1.6)
Linezolid	183 (100)	-	-
Oxacillin	181 (98.9)	2 (1.1)	-
Rifampin	183 (100)	-	-
TMP-SMX [b]	183 (100)	-	-
Vancomycin	183 (100)	-	-
Gentamicin	177 (96.7)	6 (3.3)	-
Levofloxacin	182 (99.5)	1 (0.5)	-
Ceftaroline	183 (100)	-	-

a. 6.6% of the samples showed inducible resistance to clindamycin
b. TMP-SMX, trimethoprim-sulfamethoxazole

Risk factors associated with baseline nasal colonization

The known risk factors for nasal colonization with *Staphylococcus aureus* include the previous use of antibiotics, having being exposed to healthcare settings, gender, crowding conditions, and geographic region. In our case, we built a logistic regression model including the following variables based on our literature review: use of antibiotics during the previous year (Yes/No), hospitalizations during the previous year (Yes/No), diagnosis of SSTIs during the previous year (Yes/No), smoking status (No smoker, past smoker and current smoker), and presence of respiratory diseases (Yes/No). Gender, base of recruitment, and time of service in years were included and evaluated as confounders in the model. In order to build the model, we excluded all those cases where any of these variables had a missing value or unknown answer, ending up with 638 participants. We recognized that excluding all missing categories for these variables is a very conservative option for the analysis. It was our intention to include age as a confounding variable, but it was highly correlated with time of service (rho coefficient: 0.91); therefore, we decided to use the later. Also, use of corticosteroids was not used because it is more associated with healthcare-associated MRSA, which is not the focus of this research. The variable "respiratory disease" was added if any of the participants reported any of these diseases: asthma, chronic obstructive pulmonary disease and chronic bronchitis. Given that these conditions have acute exacerbations that can lead to hospitalization, use of antibiotics, corticosteroids and smoking is involved in their pathophysiology; we evaluated interaction terms between respiratory diseases and use of antibiotics, hospitalizations, use of corticosteroids and smoking status which were included in the logistic regression model, but none of them were statistically significant so they were not included in the final model.

The adjusted odds ratio for baseline nasal colonization shows that being male increased the *Staphylococcus aureus* prevalence by 2.4 times compared with women (95% CI: 1.0 – 5.7, p=0.043). Being deployed in any other base besides Talara also increases the prevalence, showing a higher prevalence in Iquitos (OR: 2.5, 95% CI: 0.9 – 6.5, p value: 0.065), Lima (OR: 2.7, 95% CI: 0.9 – 7.3, p value: 0.051) and finally Arequipa which had 4.5 times the prevalence compared to those deployed to Talara, almost doubling the prevalence of Iquitos and Lima (95% CI: 1.7 – 11.9, p=0.002). Having respiratory disease also increased the prevalence of baseline colonization, which was 4.5 times that without a respiratory condition (95% CI: 1.4 – 14.7, p= 0.014). However, time of service had a minimal protective effect (OR: 0.97, 95% CI: 0.94 – 0.99, p=0.030). See Table 7.

The use of antibiotics during the previous year increased the colonization prevalence (OR: 1.4, 95% CI: 0.8 – 2.6), while having been diagnosed with SSTIs during the last year was associated with a lower prevalence (OR: 0.4, 95% CI: 0.1 – 1.8); but this was not statistically significant. Being hospitalized did not have any effect on the prevalence of being colonized with *Staphylococcus aureus* (OR: 1.0, 95% CI: 0.4 – 2.4). Therefore, none of the previously reported risk factors had an effect on the risk of being colonized in our study population. We performed a goodness of fit test (Hosmer and Lemeshow test), which showed that our model has a good fit (p= 0.371).

Table 7. Potential risk factors associated with baseline nasal colonization with *Staphylococcus aureus*

Variable	Unadjusted OR	Adjusted OR (95% CI)	P-value
Use of antibiotics during the previous year			
No	Ref	Ref	
Yes	1.2	1.4 (0.8 – 2.6)	0.283
Hospitalization during the previous year			
No	Ref	Ref	
Yes	1.0	1.0 (0.4 – 2.4)	0.980
Diagnosis of SSTI during the previous year			
No	Ref	Ref	
Yes	0.5	0.4 (0.1 – 1.8)	0.237
Sex			
Female	Ref	Ref	
Male	1.8	2.4 (1.0 – 5.7)	0.043
Base of recruitment			
Talara	Ref	Ref	
Iquitos	2.5	2.5 (0.9 – 6.5)	0.065
Lima	2.8	2.7 (0.9 – 7.3)	0.051
Arequipa	3.9	4.5 (1.7 – 11.9)	0.002
Smoking status			
Never smoked	Ref	Ref	
Past smoker	1.7	1.6 (0.8 – 3.5)	0.204
Current smoker	1.2	1.1 (0.6 – 2.1)	0.756
Respiratory diseases			
No	Ref	Ref	
Yes	3.5	4.5 (1.4 – 14.7)	0.014
Time of service	0.99	0.97 (0.94 – 0.99)	0.030

Change in nasal colonization status

After 1 year of follow-up, 484 participants provided two samples. Of them, 390 provided the samples after 6 months, while 94 provided the second sample after 1 year. The analysis was performed at each point of time (after 6 months and after 1 year), and as

overall. Among those with a difference of 6 months between the samples, the baseline nasal colonization prevalence was 10.8%, and the incidence (change from negative at baseline to positive in the second sample) was 10.6%, while the clearance (change from positive at baseline to negative in the second sample) was 50%. There was a significant statistical difference between the baseline and follow-up proportions of nasal colonization in the group with 6 months of difference between both samples (McNemar's test, p= 0.048). See Table 8.

Table 8. Change in nasal colonization status after 6 months of follow-up

		Nasal colonization at the second sample		
		Positive (%)	**Negative (%)**	**Total**
Nasal colonization at the first sample	**Positive (%)**	21 (50%)	21 (50%)	42
	Negative (%)	37 (10.6%)	311 (89.4%)	348
	Total	58	332	390

Among the 94 participants who provided only two samples with a difference of one year between each of them, the baseline nasal colonization prevalence was 9.6%. However, there was an increase in the incidence of nasal colonization at the second sample (14.1%), while the clearance was 55.6%. In this case, there was no significant difference between the baseline and follow-up nasal colonization (p=0.143). See Table 9.

Table 9. Change of nasal colonization status after 1 year of follow-up

		Nasal colonization at the second sample		
		Positive (%)	**Negative (%)**	**Total**
Nasal colonization at the first sample	**Positive (%)**	4 (44.4%)	5 (55.6%)	9
	Negative (%)	12 (14.1%)	73 (85.9%)	85
	Total	16	78	94

When we analyzed the total change in nasal colonization, among those who provided two samples over the complete period of follow-up, we found that the incidence rate was 11.2% while the clearance rate was 51%. See Table 10. We used the McNemar's test for paired proportions and found that there was a statistically significant difference between the rates of baseline nasal colonization and the follow-up nasal colonization (p=0.011).

484 participants provided two samples during the entire period of follow-up (390 participants did it after 6 months and 94 after 1 year). We defined change in nasal colonization status as three categories (No change, "positive to negative" and "negative to positive"). From the total group with samples at two points in time, 84.5% of the participants remained without any change in the colonization status; 10.1% changed from negative at baseline to positive at follow-up and only 5.4% cleared the baseline colonization.

Table 10. Overall change of nasal colonization status

		Nasal colonization at the second sample		
		Positive (%)	**Negative (%)**	**Total**
Nasal colonization at the first sample	**Positive (%)**	25 (49%)	26 (51%)	51
	Negative (%)	49 (11.2%)	384 (88.7%)	433
	Total	74	410	484

When we analyzed the association between the change in nasal colonization status and some demographic and other variables assessed at the follow-up we found that in the case of those who become colonized (incident cases), the highest proportion occurred among those younger than 40 years (22.1%), and also between women compared with men (15.6%). In terms of rank, the proportions were similar, being around 10% for officers, non-commissioned officers and troops. Lima had the highest proportion of acquisition (12.4%) followed by Arequipa (11.8%). Regarding the time of service, the proportion of acquisition was similar among the three groups, being approximately 10% for each of them. When we asked the participants for known risk factors associated with nasal colonization, 7.3% of those with a change from negative to positive answered "yes" to the use of antibiotics during the time of follow-up; 11.7% denied having used corticosteroids and only 1.9% reported having used corticosteroids during the follow-up period; and 11.6% had a diagnosis of skin and soft tissue infections during this period. There was a slight difference in the change of colonization status among those who were mobilized from their bases (11.4%) during the follow-up compared to those who were not (9.3%, p value: 0.662).

Those who cleared the colonization, (change from positive to negative) constituted a small fraction of the study population (5.4%). In this group, the highest proportion was among those between 40 to 49 years (7.4%), there was a slight difference in terms of

gender, male had 5.7%, while female were 4.2%. The troops had the greatest percent of clearance (7%) compared with the other two categories. The clearance was higher at Arequipa (8.2%) compared to other bases. Those with 11 to 20 years of service had the lowest percent of clearance (1.4%). In terms of known risk factors for colonization, there was no difference among those who used antibiotics during the follow-up time and those who did not report it. However, those who reported the use of corticosteroids had a clearance rate of 9.4%. No difference was observed between those who had a diagnosis of SSTI (4.5%) and those who did not (5.2%). Regarding the mobilization from the base of recruitment during the period of follow-up, the rate of clearance was similar among those who reported it (4.0%) and those who did not (6.5%). See Table 11.

Table 11. Change in nasal colonization

Variable	**Change in nasal colonization**			**P value**
	No change (%)	**Positive to negative (%)**	**Negative to positive (%)**	
Age				
18 – 29 years	236 (83.7%)	17 (6.0%)	29 (10.3%)	0.644
30 – 39 years	59 (86.8%)	1 (1.5%)	8 (11.8%)	
40 – 49 years	78 (83.0%)	7 (7.4%)	9 (9.6%)	
50 years or more	36 (90.0%)	1 (2.5%)	3 (7.5%)	
Sex				
Female	77 (80.2%)	4 (4.2%)	15 (15.6%)	0.125
Male	332 (85.6%)	22 (5.7%)	34 (8.8%)	
Rank				
Officers	48 (84.2%)	3 (5.3%)	6 (10.5%)	0.826
Non-Commissioned Officers	219 (85.5%)	11 (4.3%)	26 (10.2%)	
Troops	142 (83.0%)	12 (7.0%)	17 (9.9%)	
Base of recruitment				
Iquitos	141 (86.5%)	9 (5.5%)	13 (8.0%)	0.504
Arequipa	88 (80.0%)	9 (8.2%)	13 (11.8%)	

Talara	100 (87.7%)	3 (2.6%)	11 (9.6%)	
Lima	80 (82.5%)	5 (5.2%)	12 (12.4%)	
Time of service				
10 years or less	247 (84.0%)	18 (6.1%)	29 (9.9%)	0.635
11 – 20 years	60 (87.0%)	1 (1.4%)	8 (11.6%)	
21 years or more	102 (84.3%)	7 (5.8%)	12 (9.9%)	
Use of antibiotics during the period of follow-up				
No	244 (82.7%)	17 (5.8%)	34 (11.5%)	0.362
Yes	130 (86.7%)	9 (6.0%)	11 (7.3%)	
Unknown	35 (89.7%)	0 (0.0%)	4 (10.3%)	
Use of corticosteroids during the period of follow-up				
No	312 (83.0%)	20 (5.3%)	44 (11.7%)	0.080
Yes	47 (88.7%)	5 (9.4%)	1 (1.9%)	
Unknown	50 (90.9%)	1 (1.8%)	4 (7.3%)	
Diagnosis of SSTIs during the period of follow-up				
No	375 (84.5%)	23 (5.2%)	46 (10.4%)	0.492
Yes	18 (81.8%)	1 (4.5%)	3 (13.6%)	
Unknown	15 (88.2%)	2 (11.8%)	0 (0.0%)	
Mobilization from the base during the time of follow-up				
No	235 (84.2%)	18 (6.5%)	26 (9.3%)	0.662
Yes	171 (84.7%)	8 (4.0%)	23 (11.4%)	
No answer	3 (100.0%)	0 (0.0%)	0 (0.0%)	

Risk factors associated with change in nasal colonization status over time

In order to assess the risk factors associated with the change in the nasal colonization status over time, we performed a multinomial logistic regression, using as reference the category "No change".". We tested the following variables for inclusion in the

model: use of antibiotics during the follow-up period (Yes/No), use of corticosteroids (Yes/No), skin and soft tissue infections during the follow-up period (Yes/No). Also, these variables were evaluated for confounding: sex (Female/Male), base of recruitment (Iquitos, Arequipa, Talara, Lima), and deployments during the last year (Yes/No).

In the case of change of colonization status, in the study population with at least two samples, men compared to women are less likely to have been colonized during the follow-up than to remain without change in the colonization status (OR: 0.5, 95% CI: 0.2 – 1.0) and more likely to have cleared the nasal colonization (OR: 1.5, 95% CI: 0.5 – 4.6) than to experience no change. This situation is similar in the case of those who use corticosteroids during the time of follow-up, they had less chances of colonization (OR: 0.2, 95% CI: 0.02 – 1.4) and more chance of clearing it (OR: 1.9, 95% CI: 0.6 – 5.9). For those who used antibiotics, they had 0.8 times less risk of becoming colonized than to remain unchanged, and 1.9 more risk of clearing the colonization than to remain without change.

On the contrary, those who were diagnosed with SSTIs during the period of follow-up were slightly less likely to clear colonization compared to remaining without any change (OR: 0.9, 95% CI: 0.1 – 7.8); but their chances of being colonized were greater than to stay unchanged (OR: 1.4, 95% CI: 0.4 – 5.1). These results are similar in those who were mobilized to other bases during the follow-up. The chances of clearance compared to remaining with no change were reduced 0.4 times, but being colonized increased 1.4 times. With regard to the place of recruitment, only Iquitos showed an opposite effect (OR: 1.9, 95% CI: 0.5 – 7.4 for being cleared of nasal colonization vs. OR: 0.9, 95% CI: 0.4 – 2.3 for being colonized); but at Lima and Arequipa, the results are contradictory, because staying

at both places increased the risk of being colonized and also increased the chances of being cleared of the infection (See Table 12).

Table 12. Risk factors associated to the change in the nasal colonization status

	Clearance of nasal colonization with *S. aureus* during the follow-up		**Colonization with *S. aureus* during the follow-up**	
Variable	**Adjusted OR (95% CI)**	**P value**	**Adjusted OR (95% CI)**	**P value**
Use of antibiotics during the period of follow-up				
No	Ref		Ref	
Yes	1.1 (0.4 – 2.7)	0.895	0.7 (0.3 – 1.4)	0.324
Use of corticosteroids during the period of follow-up				
No	Ref		Ref	
Yes	1.9 (0.6 – 5.9)	0.251	0.2 (0.02 – 1.4)	0.095
Diagnosis of SSTIs during the period of follow-up				
No	Ref		Ref	
Yes	0.9 (0.1 – 7.8)	0.951	1.4 (0.4 – 5.1)	0.640
Sex				
Female	Ref		Ref	
Male	1.5 (0.5 – 4.6)	0.515	0.5 (0.2 – 1.0)	0.054
Place of recruitment				
Talara	Ref		Ref	
Iquitos	1.9 (0.5 – 7.4)	0.340	0.9 (0.4 – 2.3)	0.966
Lima	2.0 (0.5 – 8.9)	0.071	1.6 (0.6 – 3.9)	0.313
Arequipa	3.5 (0.9 – 13.3)	0.355	1.2 (0.5 – 2.9)	0.654
Mobilization to other bases				
No	Ref		Ref	
Yes	0.6 (0.2 – 1.4)	0.194	1.4 (0.7 – 2.6)	0.309

The previous analysis used as reference category "No change" (it included those participants who remained negative or positive in all the three samples they provided). When we repeated the analysis, using as reference category with only those participants who remained negative during the study period, we found very similar results as to those shown in Table 12. There were no changes in magnitude or direction of the associations found, although being deployed to Arequipa now became statistically significant for increasing the clearance of nasal colonization (OR: 3.9, 95% CI: 1.0 – 15.3, p value: 0.047). See Table 13.

Table 13. Risk factors associated to the change in the nasal colonization status (Negative as reference level)

	Clearance of nasal colonization with *S. aureus* during the follow-up		**Colonization with *S. aureus* during the follow-up**	
Variable	**Adjusted OR (95% CI)**	**P value**	**Adjusted OR (95% CI)**	**P value**
Use of antibiotics during the period of follow-up				
No	Ref		Ref	
Yes	1.0 (0.4 – 2.7)	0.948	0.7 (0.3 – 1.4)	0.287
Use of corticosteroids during the period of follow-up				
No	Ref		Ref	
Yes	1.9 (0.6 – 5.8)	0.288	0.2 (0.02 – 1.3)	0.090
Diagnosis of SSTIs during the period of follow-up				
No	Ref		Ref	
Yes	0.9 (0.1 – 7.9)	0.965	1.5 (0.4 – 5.5)	0.577

Sex				
Female	Ref		Ref	
Male	1.5 (0.5 – 4.9)	0.460	0.5 (0.2 – 1.0)	0.065
Place of recruitment				
Talara	Ref		Ref	
Iquitos	2.0 (0.5 – 7.8)	0.301	1.0 (0.4 – 2.4)	0.960
Lima	2.2 (0.5 – 9.5)	0.398	1.7 (0.7 – 4.2)	0.270
Arequipa	3.9 (1.0 – 15.3)	0.047	1.4 (0.6 – 3.3)	0.486
Mobilization to other bases				
No	Ref		Ref	
Yes	0.5 (0.2 – 1.3)	0.165	1.4 (0.7 – 2.6)	0.312

Loss of participants

As was indicated above, we had a lost to follow-up rate of 35.9% when we analyzed the information from participants with at least 2 samples. We compared the baseline characteristics between the participants who continued in the study and provided two samples, and those who stopped their participation for any reason after they provided the baseline sample. See Table 14. Regarding some demographic variables, we lost men and women in similar proportions (34.2% vs. 36.4%); in terms of age groups, we lost more participants between 18 – 29 years (37.3%) and 40 -49 years (37.7%). The proportions of lost to follow-up were very similar among the three categories of time of service (above 30%); and we also lost similar numbers if we separated the year of enrollment (2013: 35.7% vs. 2014: 37.6%). For all these variables, we did not find any statistically significant difference. However, we found a difference when we compared the two groups by rank and base of recruitment. We lost more troops (43.8%) than officers (32.1%) and non-commissioned officers (30.4%) (p=0.001). We lost more participants at Lima (42.5%), while at the other study sites, our losses ranged from 29.6% to 35.6% (p value: 0.018).

When we compared the clinical characteristics of the participants; of those who were lost to follow-up, 42.0% had being hospitalized the previous year, 25% had reported being diagnosed with any type of SSTI; and 40% reported a respiratory disease. There were not statistically significant t differences between those who continued the in the study and those who were lost to follow-up. We found statistically significant differences for use of antibiotics during the previous year, smoking status and use of corticosteroids. The biggest difference in the use of antibiotics was in the group that did not provide an answer or did not know if they had used these medications, 54.5% were lost to follow-up; in terms of smoking status, 41.7% of those who reported current smoking habits were lost to follow-up. In the case of use of corticosteroids our results are similar to those for antibiotics, a higher rate was seen in those who did not answer (46.3%).

Table 14. Characteristics of participants who were lost to follow-up

Variable	**Study participants with 2 samples**	**Lost to follow-up**	**p value**
Sex			
Female	96 (65.8)	50 (34.2)	0.627
Male	388 (63.6)	222 (36.4)	
Age categories			
18 – 29 years	282 (62.7)	168 (37.3)	0.376
30 – 39 years	68 (71.6)	27 (28.4)	
40 – 49 years	94 (62.3)	57 (37.7)	
50 or more years	40 (66.7)	20 (33.3)	
Rank			
Officer	57 (67.9)	27 (32.1)	0.001
Non-commissioned officer	256 (69.6)	112 (30.4)	
Troops	171 (56.3)	133(43.8)	
Time of service			
10 years or less	294 (63.5)	169 (36.5)	0.795
11 -20 years	69 (67.0)	34 (33.0)	
21 years or more	121 (63.7)	69 (36.3)	

Place of recruitment			
Iquitos	163 (64.4)	90 (35.6)	0.018
Arequipa	110 (67.1)	54 (32.9)	
Talara	114 (70.4)	48 (29.6)	
Lima	97 (54.8)	80 (42.5)	
Respiratory diseases			
No	472 (64.1)	264 (35.9)	0.704
Yes	12 (60.0)	8 (40.0)	
Smoking status			
Never	232 (67.2)	113 (32.8)	0.045
Past	86 (68.3)	40 (31.7)	
Current	151 (58.3)	108 (41.7)	
Use of antibiotics during the previous year			
No	271 (63.5)	156 (63.5)	0.001
Yes	183 (69.6)	80 (30.4)	
Unknown	30 (45.5)	36 (54.5)	
Use of corticosteroids during the previous years			
No	340 (63.4)	196 (36.6)	0.049
Yes	108 (70.6)	45 (29.4)	
Unknown	36 (53.7)	31 (46.3)	
Hospitalized during the previous year			
No	420 (65.1)	225 (34.9)	0.316
Yes	51 (58.0)	37 (42.0)	
Unknown	13 (56.5)	10 (43.5)	
Diagnosis of SSTI during the previous year			
No	428 (63.8)	243 (36.2)	0.179
Yes	33 (75.0)	11 (25.0)	
Unknown	23 (56.1)	18 (43.9)	

Carrier index

We calculated the carrier index in the 186 participants who provided three samples. The carrier index is defined as the total number of positive samples/total number of samples provided. The resultant number is categorized and we obtained 4 groups: No carrier (carrier index = 0), Occasional carrier (0.1 – 0.4), Intermittent carrier (0.5 – 0.8), and Persistent carrier (0.9 – 1). Among those who provided three samples, 70.4% (n = 131) were no carriers, meaning that they were negative at each time of sampling; 18.8% (n = 35) were occasional carriers (1 positive of 3 samples), 6.5 (n = 12) were intermittent carriers (2 positive of 3 samples), and only 4.3% (n = 8) were persistent carriers (all samples were positive). These 8 persistent carriers were male, 4 were at Arequipa, 2 at Iquitos and 1 at Lima and Talara. Most of them (5) had reported use of antibiotics during the previous year, but only 1 reported use of corticosteroids, and 1 reported being hospitalized the previous year. None of them had been diagnosed the year before with any SSTI.

Reproducibility Analysis

We took duplicate samples in 123 participants in order to evaluate the variability of the lab tests at each location (Lima, Chiclayo, Arequipa, and Iquitos) and sampling time (Baseline, 6 months and 1 year) for detecting *Staphylococcus aureus*. We calculated the overall positive percent agreement (PPA); and then we did a sub-analysis by time of sampling, by person who took the sample and by base of precedence. The overall positive percent agreement was 0.68 (See Table 15).

Table 15. Comparison between first and second repeated samples (N=123)

		Second sample		
		Positive (%)	**Negative (%)**	**Total**
First sample	**Positive (%)**	15 (83.3%)	3 (16.7%)	18 (14.6%)
	Negative (%)	11 (10.5%)	94 (89.5%)	105
	Total	26 (21.1%)	97	123

When analyzed by time of sampling, those samples taken at baseline (October – November 2013) had a PPA of 0.73, while the PPA corresponding to the 6 month visit (April – August 2014) decreased to 0.40. At the one-year visit (October – December 2014) the PPA (0.71) increased to similar levels as those at the baseline visit. In terms of the person taking the nasal swabs, we had three operators; operators 1 and 2 had similar PPAs (0.69 vs. 0.67), respectively); operator 3 had perfect agreement (1.0) but this person only participated in few visits (9 samples, at 3 locations, during 2 visits). Regarding the base of recruitment, Arequipa and Lima had the highest PPA (0.92 vs. 0.86, respectively), followed by Talara (0.50) and Iquitos with the lowest PPA (0.22).

To evaluate the possibility of variability between time periods and locations, we created 54 quality control nasal swabs, which were labeled and shipped to the lab, along with the regular swabs. All these 54 "created" samples were *Staphylococcus aureus* positive, and therefore identical, reaching an agreement of 100%. Based on these results, the lab procedures are reliable; and it is possible that the disagreements are more related to taking the samples and the procedures prior to the shipping of the nasal swabs to the lab.

OBJECTIVE 2: SKIN AND SOFT TISSUE INFECTION SURVEILLANCE

Skin and soft tissue infections (SSTI) are the main clinical event associated with MRSA infections; they impose a heavy burden on the military health system worldwide and affect the readiness of personnel to carry out military operations. Lack of information about these diseases is a critical fault in the Peruvian military health system, so we included the surveillance of these events in the current electronic surveillance system of infectious disease of the Peruvian Air Force in order to obtain baseline estimates of SSTIs.

Prevalence rates

We collected all the SSTI cases diagnosed at each health facility of the Peruvian Air Force from 2012 to 2014. Data is complete for years 2012 and 2013. For 2014, we used information through September for 25 of 27 bases (the missing 2 health facilities are the two largest hospitals in Lima). In order to collect the data, we visited each health facility, and reviewed the sources of information available at the time of the visit (log-books, prescription receipts, notebooks, and available datasets). The Peruvian Air Force requires that each health facility complete an official log-book (See Figure 9) with numbered pages; the health personnel charged with this activity must record the serial number, name, age, rank, sex, date of diagnosis, diagnosis, ICD-10 code, diagnostic tests, and treatment. However, a log-book with these characteristics was found only in 1 of 27 bases (3.7%). At the rest of the bases, the information was recorded manually in unofficial notebooks (see Figure 10).

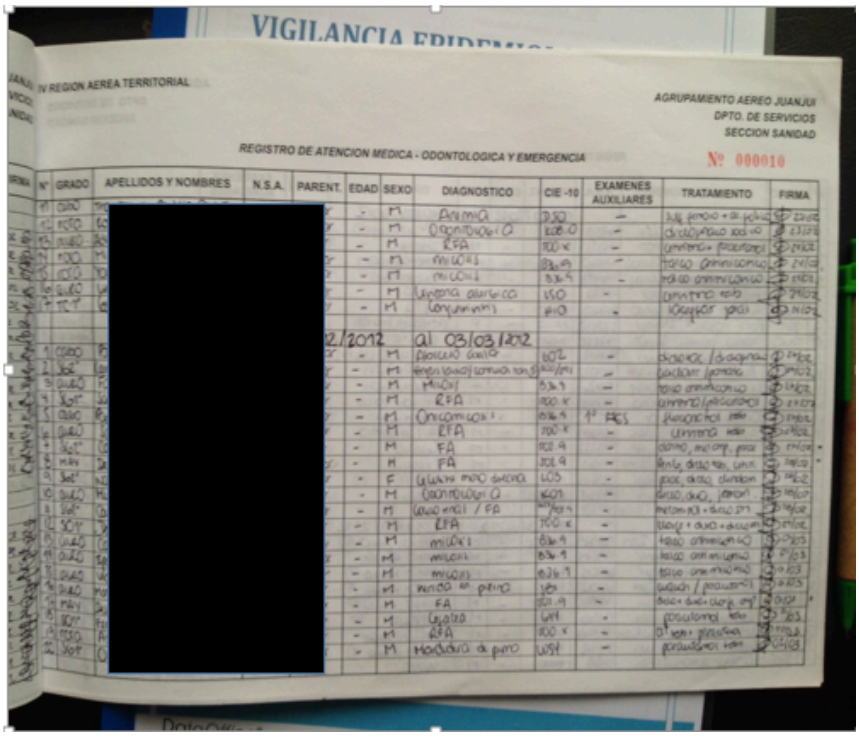


Figure 9. Official log-book from the Peruvian Air Force with all the variables asked for the Directorate of Health

The information recorded for this documentation comes from the register sheets completed by the physicians during the medical visit. Later, health personnel transcribe this information into the log-books. Another source of information was the copy of prescriptions (See Figure 11), and in three health facilities, they had electronic records of the medical encounters, which include medical diagnosis, laboratory orders and prescriptions. In order to collect all the SSTI cases diagnosed at each year, we reviewed all the available sources of information at each site.

We defined a SSTI as any written diagnosis of impetigo, ecthyma, erysipelas, folliculitis, furunculosis, abscess, infected wound (excluding surgical wounds), cellulitis, pyoderma, and lymphadenitis. In the absence of the written diagnosis, we used the

following ICD-10 codes: L01, L02, L03, L04, L08, and L73. For each identified case we recorded the age, rank (Officers, Cadets, Non-Commissioned Officers (NCO), NCO in training, and troops), date of diagnosis, diagnosis, ICD-10 code and treatment provided.

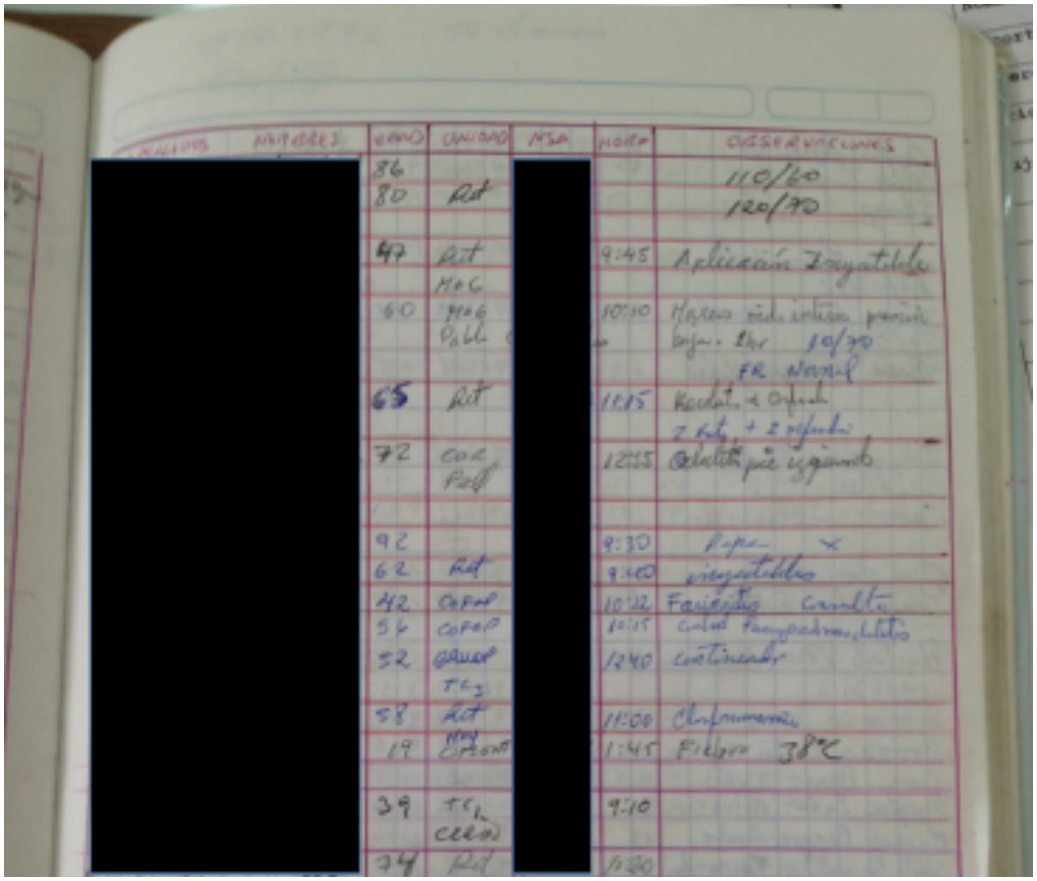


Figure 10. Unofficial log-book from the Peruvian Air Force

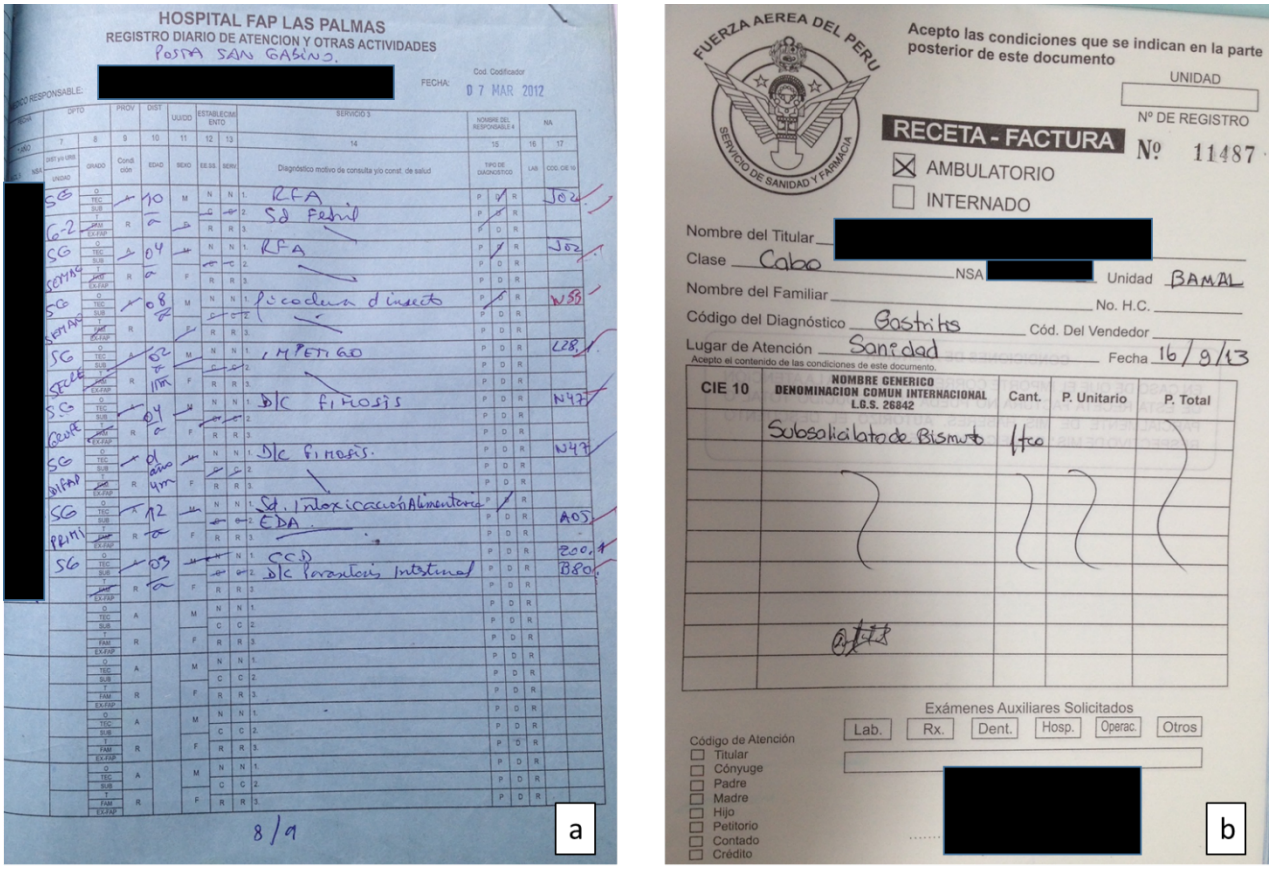


Figure 11. (a) Daily record sheets and (b) Prescription receipts

In order to calculate the prevalence of SSTIs, we used the approximate numbers of the total active duty military personnel of the Peruvian Air Force in year 2014, disaggregated by sex and rank. These values were approximate due to national security reasons and were almost the same by each year. Therefore, our total population included 10, 800 active duty personnel, of them, 1,890 were female and 8,910 were male. Regarding the rank, 1,663 were officers, 281 were cadets, 6,167 were non-commissioned officers (NCO), 507 were NCOs in training and 2,182 were troops.

During the period 2012 to 2014, there were 1,836 cases of SSTIs registered at the 27 health facilities of the Peruvian Air Force (however for 2014 we only included information form 25 health facilities). Therefore, the cumulative prevalence of SSTIs was 17.0 %. When we analyze the prevalence by each year (See Table 16), the prevalence for 2012 (7.9%, 95% CI: [7.4 – 8.4]) and 2013 (5.8% 95% CI: [5.3 – 6.3]) were similar. However our estimate of prevalence in 2014, missing the cases in the numerator from the two hospitals in Lima, was lower (3.3%, 95% CI: [2.9 – 3.6]).]). See Figure 12.

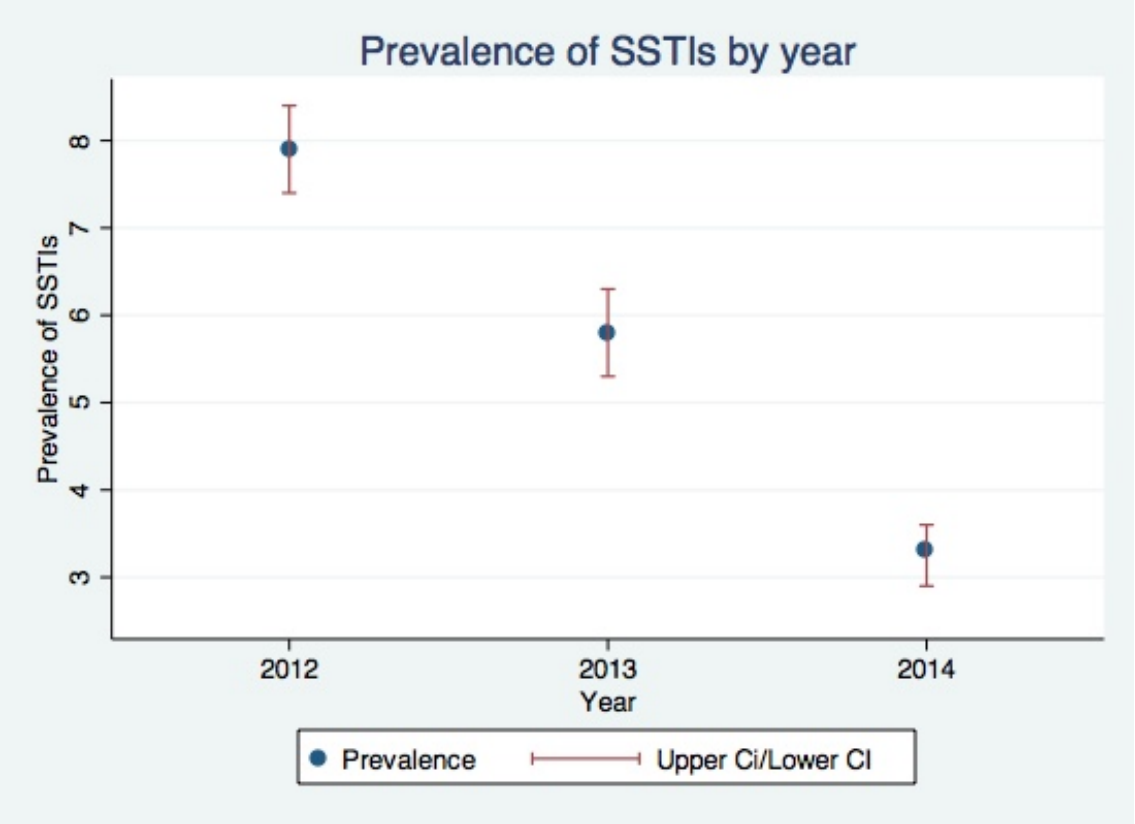


Figure 12. Prevalence rates of SSTIs by year (2012 – 2014)

In the case of gender, men are more affected at each year compared with women; although for 2014 the rate in this group was 23%, doubling the rate of 2013 (11.6%).

Regarding the ranks, non-commissioned officers were the group with the highest prevalence rates across each year, followed by the troops. However, when compared between each year, there was an increase of approximately 10 percentage points between 2012 and 2013 for both groups. On the other hand, the cadets and NCO in training have shown a decline in the prevalence rates since 2012 (from 11.0% to 0.3% for the cadets, and from 23.7% to 8.7% for NCO in training). In terms of region, the highest prevalence corresponds to the central region (Central Coast and Highlands), followed by the other three regions that possess less numbers of active military duty personnel. In 2014, our estimate of the prevalence of SSTIs in the central region was much lower, but it might be due to the fact that we did not collect information from the two hospitals at Lima for 2014.

Table 16. Proportions of specific SSTIs per each year

	Year		
Variable	**2012 (%)**	**2013 (%)**	**2014* (%)**
Sex			
Female	98 (11.6%)	96 (15.2%)	82 (23.0%)
Male	747 (88.4%)	534 (84.8%)	274 (77.0%)
Rank			
Officer	93 (11.0%)	60 (9.5%)	44 (12.4%)
Cadet	93 (11.0%)	24 (3.8%)	1 (0.3%)
NCO	355 (41.9%)	327 (51.9%)	31 (8.7%)
NCO in training	201 (23.7%)	51 (8.1%)	31 (8.7%)
Troops	106 (12.5%)	168 (26.7%)	89 (25.0%)
Region			
North	75 (8.8%)	95 (15.1%)	91 (25.6%)
Center	586 (68.9%)	361 (57.3%)	135 (37.9%)
South	89 (10.5%)	103 (16.3%)	72 (20.2%)
East	100 (11.8%)	71 (11.3%)	58 (16.3%)
TOTAL	2543 (100%)	1890 (100%)	880 (100%)

* Numbers for 2014 are missing data from two large hospitals in Lima

The most common diagnosed skin and soft tissue infection during the period 2012 – 2014 was cellulitis (n = 958, 52.2%), followed by abscess (n = 434, 23.6%) and pyoderma (n = 219, 11.9%), while acute lymphadenitis, (n = 17), folliculitis (n = 85) and impetigo (n = 123) were 12.3% together. When analyzed by year, 2012 was the year where the 46.3% of the diagnoses occurred, and then we observe a decline in the proportions, reaching 19.4% in 2014 (although we did not include information from the two big hospitals at Lima, which can explain this difference). Cellulitis was the more common diagnosis each year, accounting for 61.0% of reports in 2013 but only 46.6% of reports in 2014. Abscess were more often diagnosed in 2014 (31.7% of SSTIs) as well as pyoderma (%). These two diseases increased their proportion of total SSTIs over time (See Table 17).

Table 17. SSTI diagnosis during the period of study

Disease	**Number of cases (%) per year**			**Total**
	2012	**2013**	**2014**	
Cellulitis	408 (48.0)	384 (61.0)	166 (46.6)	958 (52.2)
Abscess	181 (21.3)	140 (22.2)	113 (31.7)	434 (23.6)
Pyoderma	102 (12.0)	64 (10.2)	53 (14.9)	219 (11.9)
Impetigo	82 (9.6)	27 (4.3)	14 (3.9)	123 (6.7)
Folliculitis	72 (8.5)	7 (1.1)	6 (1.7)	85 (4.6)
Acute lymphadenitis	5 (0.6)	8 (1.3)	4 (1.1)	17 (0.9)
Total	850 (100)	630 (100)	356 (100)	1836 (100)

As was mentioned above, the identification of SSTI cases was based on the review of different sources of information, looking for the written diagnosis or the ICD-10 code. In 34.8% of the cases, we did not find the written diagnosis, while in only 4.2% the ICD-10 was absent. The prescribed antibiotics were not included in many of the log-books, with only 7.3% of the records (134 of 1836) having information about the prescribed

antimicrobial treatment. Dicloxacillin was prescribed most of the time as monotherapy (52.2%) or in combination with other antibiotics (11.2%). The remaining 36.6% corresponds to multiple drug combinations, which include penicillin, amikacin, ciprofloxacin, clindamycin, amoxicillin, ceftriaxone, etc.

In Figure 13, we report the time series for the SSTI cases by month (1 represents January and 12, December) and for each of the three years. This graph represents the overall number of cases of SSTIs, including all the 27 health facilities (although for 2014 it only contains information form 25 health facilities). We can appreciate that for 2012 there was an increase in the number of cases during the first half of the year, reaching a peak in March, declining and then rising again in May. After July, there is a decline in the number of SSTIs. However, for year 2013, the number of cases during the first half of the year is almost the same until October followed by a decline in the cases. In the case of year 2014, we observe instead of a rise, a decline in February, later there is an increase that reaches its peak in May and then starts to decline in the SSTI cases, following the same trend noted in the previous years.

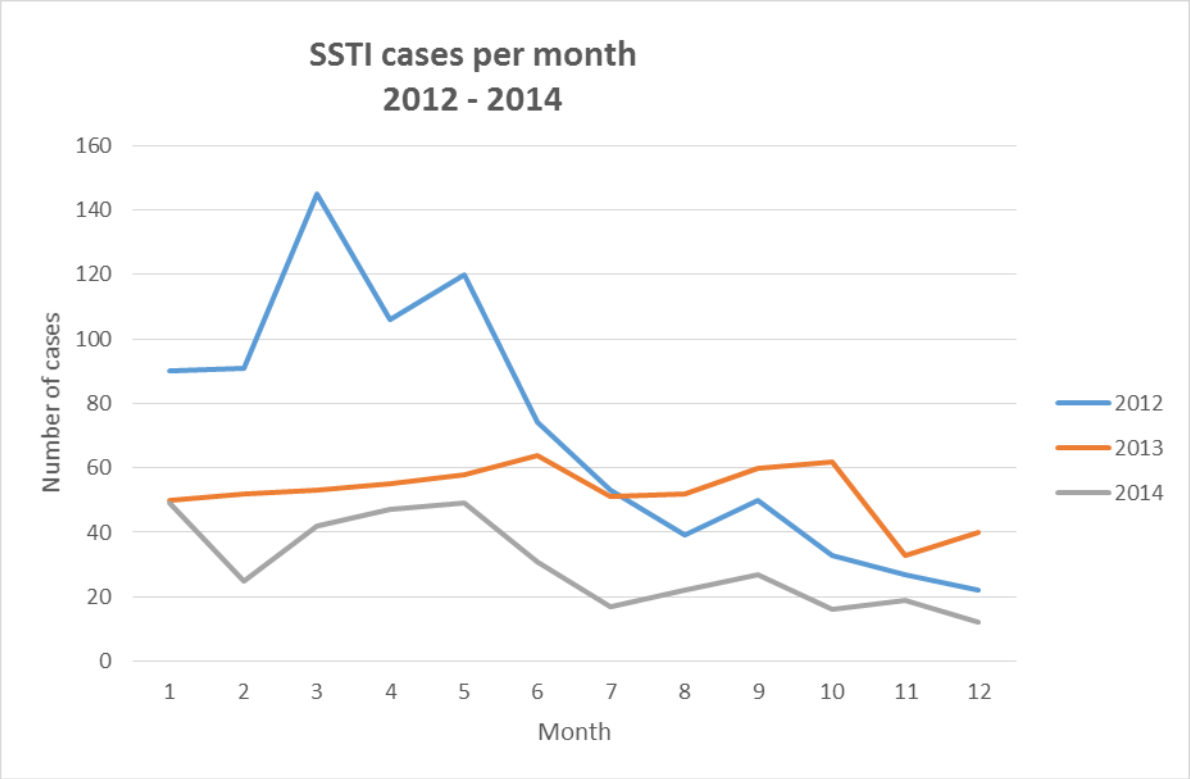


Figure 13. SSTI cases by month (January – December) and year.

When we disaggregate the cases by region, we observe that the trend changes. In the case of the northern region, we observe an increase in the number of SSTI cases during the first 4 months of the year, with a peak in March that is consistent in each year. In the case of 2012, after April, there was a decline that was deepest in May and then the cases remained in numbers per month until the end of the year. This was a similar behavior when we compared it with 2013 - 2014.

In terms of climate conditions, the northern region covers the north coast, which is characterized by a dry arid climate, with higher temperatures between December to March. This distribution matches the peaks described in the graph (See Figure 14).

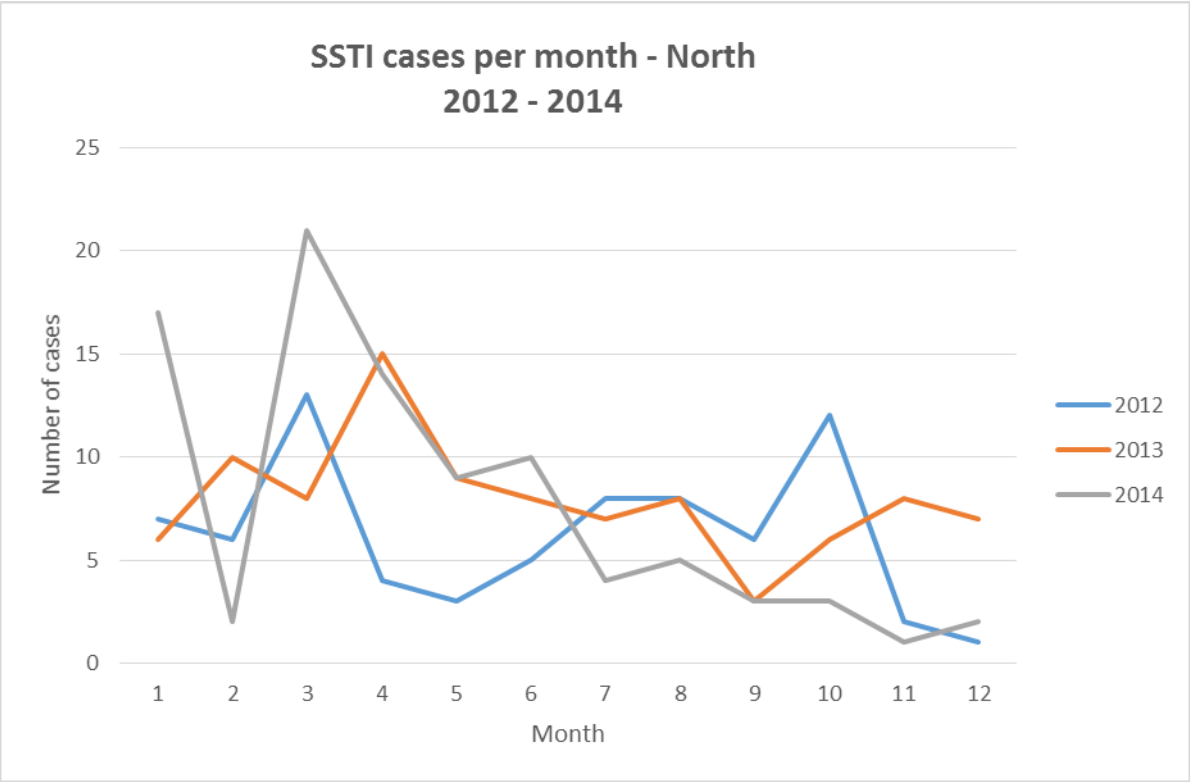


Figure 14. SSTI cases in the Northern region – 2012-2014

The central region included the two largest hospitals in Lima, and also includes most of the active duty personnel of the Peruvian Air Force. In 2012 there was a peak in the number of cases during March and May, and then it declined, similar to the other two years. On the other hand, during the first half of the year, the number of cases was low for 2013 and 2014; but then there was a slight increase by June of each year; however, the rest of the year, the number of cases remained low. In terms of climate, there is an increase in the temperatures from December to March, similar to the behavior of the northern region, but without reaching the peaks of temperature that characterizes the north (See Figure 15).

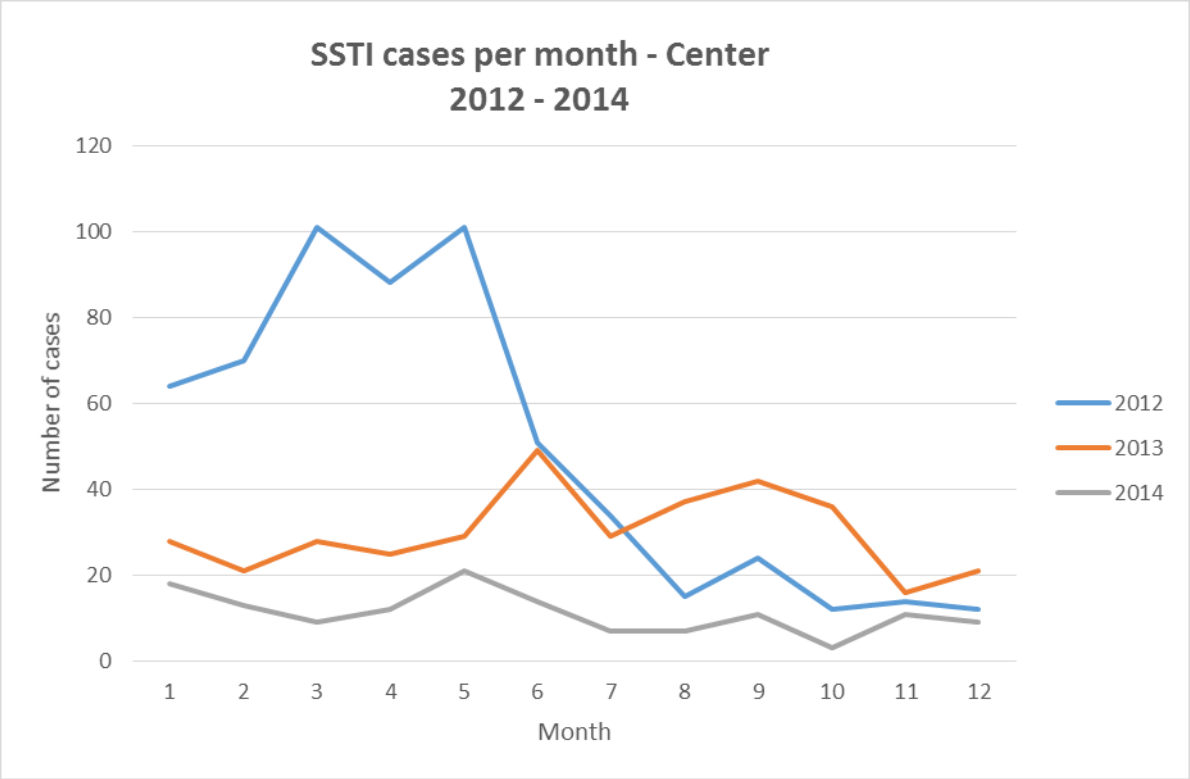


Figure 15. SSTI cases in the Central region – 2012-2014 (Note 2014 missing 2 hospitals from Lima so incomplete data included)

The southern region has 4 bases, 3 in the highlands (Arequipa), and 1 on the coast (Tacna). The range of temperatures is 10°C to 25°C during the year, with the lowest temperatures during the southern winter (June – July). In this region, there is a similar pattern for the three years; there were increases during the summer (December – March), and then a decline during the middle of the year that corresponds with the winter (See Figure 16).

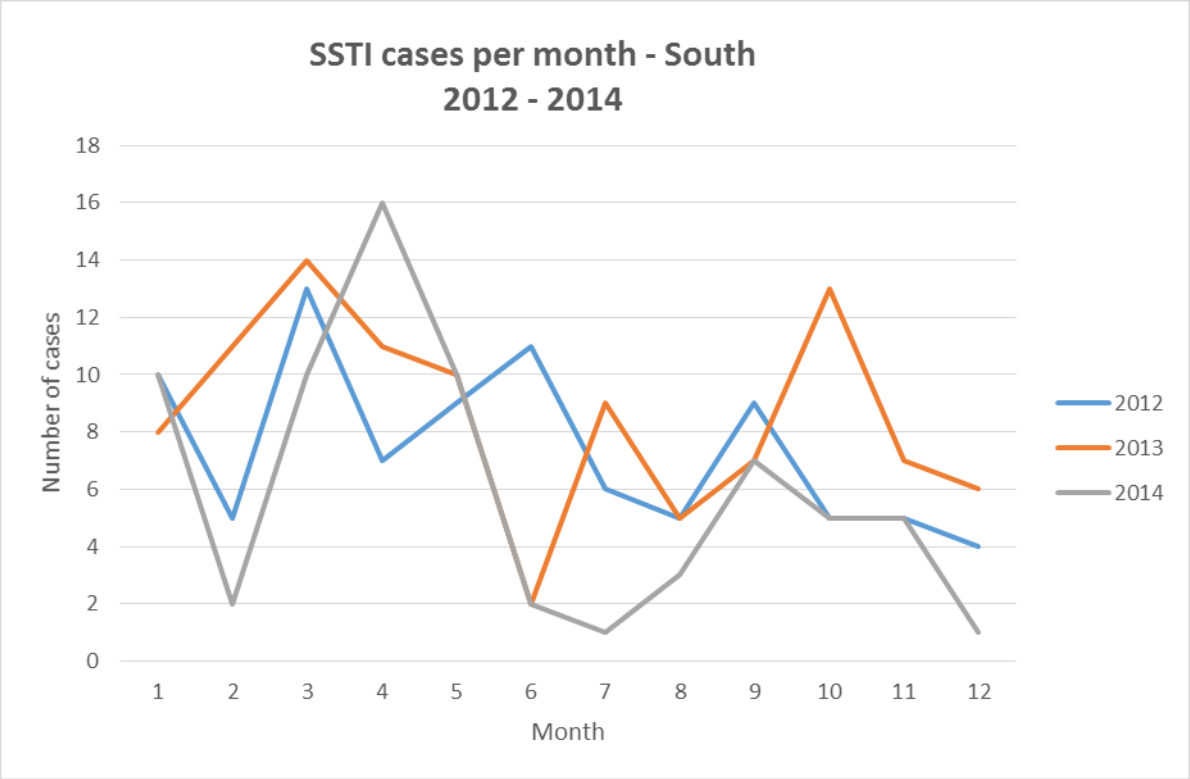


Figure 16. SSTI cases in the Southern region – 2012-2014

The Eastern region includes all the bases located in the jungle with a tropical climate, (Tarapoto, Juanjui, Iquitos, Pucallpa and Puerto Maldonado). For all these cities, the temperatures range between 25°C to 35°C on average, and there are two distinct seasons: December – April with high precipitation and higher temperatures, and May – October without precipitation and a slight decrease in the temperature. The Eastern region has 1 regional hospital located at Iquitos; and we observed a different pattern of SSTIs between 2012 and the other two years. In 2012, there were two peaks in the number of SSTI cases, one at March and the other between August and September. In the case of the 2013 and 2014, there was also an increase in the beginning of the year (February), followed by a reduction during March and April, increasing again in May, after that the cases were

fewer for the rest of 2013 although there was another peak around August – September (See Figure 17).

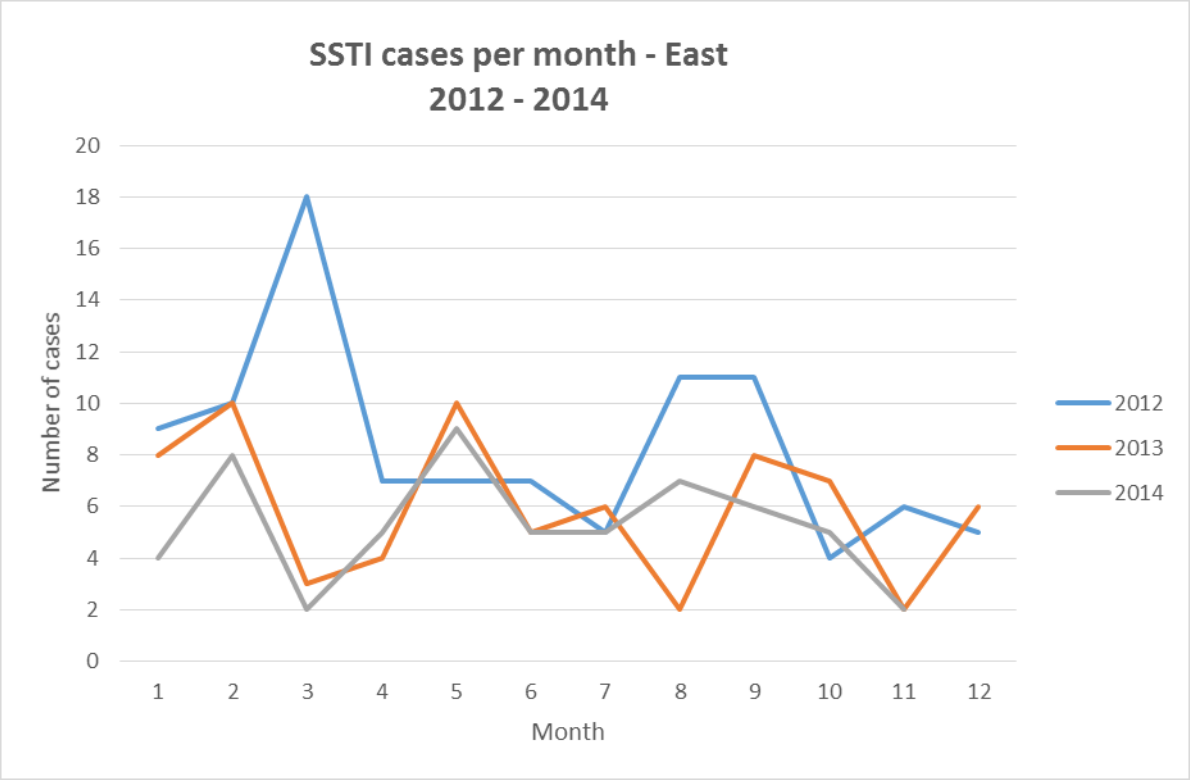


Figure 17. SSTI cases in the Eastern region – 2012-2014

Evaluation of the SSTI surveillance system

Implementation

As part of this study, we endeavored to add the report of skin and soft tissue infections to the current electronic surveillance system of infectious diseases of the Peruvian Air Force. As explained in the methods, the system relies on the scheduled report of any of the events under surveillance by trained health personnel. It receives reports by phone and Internet from every base in Peru. In order to access the system, the stakeholders

must enter a username and password, after that they complete a questionnaire with standard variables regardless of the type of media report. The diseases are divided in two groups based on the frequency of report: Collective reports are those with high numbers of cases per week (acute respiratory infections, acute diarrheal diseases and asthma), and Individual reports, which are those that occur less frequently. We decided to include the surveillance of SSTIs as an individual report. This surveillance system has functioned in the Peruvian Air Force since 2010, and there was regular training of the health personnel responsible for reporting at each facility at least twice a year. There were also online refresher courses offered on an intermittent basis. In addition, we encouraged the personnel to train their replacements in case they were mobilized to other units.

The Peruvian Air Force formally requested addition of the SSTI questionnaire in September 2013, coincident with our visits to each health facility of the Peruvian Air Force to collect SSTI cases from the log-books. However, due to technical, financial and logistical issues (which included lack of personnel to translate the questionnaire into the surveillance system datasets, insufficient funds to record the interactive voice recognition, and misunderstanding between the Air Force and NAMRU-6's Bio Informatics Unit) the inclusion of the SSTI questionnaire in the system was delayed until mid-March 2014 when the technical tests were completed, and after that it was ready to receive reports only by Internet (not by phone). We asked the personnel to start reporting into the system commencing April 2014. The main objective of the inclusion of the SSTI in the reporting system was to assess baseline rates of this event, which can affect the readiness of the military personnel for military operations.

As previously mentioned, entering the report requires prior training. Given that the method of reporting was similar to the reporting activities that the stakeholders performed for other diseases, we performed the training of the health personnel responsible for this activity from September to November 2013. The SSTI report was not available by phone or Internet at that time, and we used a file with the questionnaire that had to be completed for each stakeholder, graphs with the most common SSTIs, and a set of instructions regarding which were the SSTI diagnoses were under surveillance. Based on our previous experience with surveillance activities, we asked the administrator of the electronic surveillance system of the Peruvian Air Force to continue the retraining of the personnel every quarter in order to refresh their knowledge, as usual; and we visited each facility again in April – June 2014.

The CDC guidelines for evaluating electronic surveillance systems recommends that every evaluator must assess the usefulness of the system based on the objectives of surveillance and then evaluate the attributes of the system (simplicity, flexibility, acceptability, data quality, sensitivity, timeliness, stability and representativeness). In our case, we performed an evaluation including usefulness and attributes.

Usefulness

After 9 months, there were 229 SSTI cases in active duty personnel during the period April – December 2014 that should have been reported into the surveillance system. 16 reports were included in the system for the same period; of them, only five corresponded to active duty personnel, our study population. In this way, only 2.2% of all the reports were entered in the surveillance system. Additionally, this proportion might be even lower because the SSTI cases from two large hospitals at Lima were not included; additionally, we did not have information after October 2014 for the bases at Iquitos, Pucallpa, Piura,

Talara and Arequipa. Therefore, we were unable to assess baseline rates of SSTI with the surveillance system and the system as used was thus not useful for reporting the SSTI cases.

The five reports that were extant came from two regional Hospitals located in Arequipa (4 cases) and Iquitos (1 case). The dates of the reports extend from April to October 2014 for both hospitals, but only Arequipa reported through the whole year, while Iquitos only reported once in April. We performed an initial visit at each health facility in October – November 2013 when training for the reporting of SSTI cases took place. We revisited them on April – June 2014 in order to retrain the stakeholders and demonstrate the report via the Internet. The third and last visit occurred on January 2015 to evaluate the system after 9 months of functioning. At the time of the third visit, we knew that the report rate was low, despite the instructions from the Directorate of Health of the Peruvian Air Force regarding the obligation to send the reports to the epidemiological surveillance system. Given this situation, we informally asked about the reasons why the health personnel did not send the report despite the fact that there were SSTI cases. At the 25 health facilities where we could go and gather information (unfortunately due to lack of coordination, we could not visit the two larger hospitals at Lima), the main reasons were grouped in three categories:

a. ***No available Internet at the location***. The main reporting media is the phone, especially in units outside Lima. Unfortunately, this option was not available for the SSTI system due to the costs associated with the creation of the Interactive Voice Recording (IVR), a technical step needed for the development of the report by phone.

b. ***Lack of trained personnel to report***. The Peruvian Air Force has a mandatory change of personnel every year at all the bases. The health personnel undergo this reassignment process also. During our third visit, we found that at 87.5% (21 of 25) of the health facilities, the reporting personnel had changed, and had not been trained in how to report to the system. The health personnel included physicians doing their mandatory rural service in the Armed Forces. In this case, this service starts in May and ends in April of the following year. By the time of our evaluation, almost all of the physicians had been changed and therefore, we lost many of the reporting personnel who had been originally trained.

c. ***Lack of time to send the report.*** At the larger bases with regional hospitals, one of the reasons for not sending a report was the duplication of the activities and lack of time, because they argued that they were responsible for other activities, which included the report to the Ministry of Defense and the Ministry of Health, which is based on paper forms.

In addition, the data quality from the new reporting system was not good. Of the 5 reports entered into the system, only 1 matched the log-books of the base from where it was sent. These 5 reports came from two regional hospitals, with log-books at Emergency, Hospitalization and Outpatient services. We were able to review the emergency and outpatient services, but not those from Hospitalization and it is possible that these cases not found in the log-books corresponded to hospitalized patients.

Attributes

Simplicity

The simplicity of the system was assessed through the analysis of the steps involved in sending a report to the system. The reporter collected SSTI cases from the log-books at each health facility. After this, the personnel entered the report by Internet, completing a form of 20 questions, with only 11 of them mandatory. This process took about 3 minutes to be performed. After the report was sent, it appeared on the dataset immediately. Sending a SSTI report to the system was a simple process.

Flexibility

This attribute was measured based on the changes requested by the Peruvian Air Force to modify the SSTI system. The system only worked with the Internet and not by phone, which was the medium most available to reporters throughout the system. Due to logistic and funding issues this issue could not be solved; therefore the flexibility was rated low.

Data quality

Data quality was defined based on (1) the proportion completion of reports (number of fully completed reports/total number of reports) and (2) the error per report proportion (number of errors/total number of reports). The questionnaire that each reporter had to complete had 20 questions, but of them, 11 were mandatory in order to send the report. These were date of admission (date), health facility (Base), district (Place of precedence), type of population (officer, sub-officer, cadet, alumni, troops, or civilian), type of event (any disease under surveillance), gender (female, male), age in years, how many days ago was the symptoms onset?, is the patient alive? (Yes/No), type of SSTI (erysipelas, ecthyma,

cellulitis, impetigo, furunculosis, necrotizing fasciitis, folliculitis, Carbunculosis), and situation of the case (probable, confirmed).

The first three variables (date of admission, health facility and district) are assigned by default by the system based on the identity of the reporter. We used the other 8 variables to assess the data quality. In terms of completeness of the information, we only could compare one report that had a match with the log-books. For this unique case, the information required to send the report was complete, and it did not have any error in any of the required fields of report. But this is hardly representative of the entire system.

Sensitivity

The sensitivity of the system was assessed after 9 months and was calculated as the sensitivity of case reporting. Our gold standard was the cases reported in the log-books during the follow-up period. The sensitivity of case reporting was defined as number of cases detected by the SSTI surveillance system divided by the total number of cases recorded in the log-books. It was 2.2%, far from the expected 80% of sensitivity that is usually the cutoff value for a good sensitivity.

Timeliness

In terms of timeliness, we asked each reporter to send the report of SSTIs as soon as the case was diagnosed and we gave them up to 1 day after the diagnosis to send the report. When we checked the log of each report, the date of medical attention was the same as the day of report, indicating that the reports were sent on time. Therefore, for those completed our timeliness is 100%. Again, the low use rate hinders the ability to generalize to the rest of the system.

Acceptability

This attribute changed over time among the personnel responsible for the SSTI surveillance system at different levels. During the training of reporters, they expressed enthusiasm to perform the reporting activities. In addition, the health authorities always expressed support and willingness to continue and expand these surveillance activities. In our evaluation, despite the initial enthusiasm, the acceptability reduced over time and was manifest with the low reporting by the end of the period of evaluation.

Stability

We could not assess the stability of the system due to the lack of technical information regarding the number of malfunctions of the system. However, based on verbal reports from the administrator of the system, the system was offline on only a few occasions, but we do not have a specific measure (total time offline).

Overall, the SSTI surveillance system did not function as expected. There were several setbacks from the implementation of the system such as a delay in the delivery of the system, the report only being available via Internet when most of the time the report is sent by phone. Other factors included administrative issues such as the poor monitoring of the system due to lack of equipment and tools to perform this activity; the duplication of surveillance activities; the assignment of reporting personnel to other activities that were considered a higher priority; and the mobilization of trained health personnel to other bases that led to a drop in the surveillance report. Logistical aspects such as the lack of Internet at the health facilities and the absence of training courses for reporting into the system, also affected the performance of the SSTI surveillance system.

CHAPTER 4: Discussion

OVERVIEW OF MAJOR FINDINGS

Our findings show that nasal colonization with *Staphylococcus aureus* is lower than expected at baseline, but rises over the study period to levels consistent with rates published for the region. To our knowledge, this is the first study in a military population in Latin America and the first that assesses prevalence of nasal colonization in four different cities in Peru. A new MRSA strain (possibly related to New York/Japan clone), not previously reported in Peru, was found in the country. The risk factors for acquisition of SA in this study population were consistent with those reported in the literature. Many factors affected the performance of the SSTI surveillance system. We identified numerous challenges in implementing a disease surveillance system in this developing country setting.

OBJECTIVE 1: NASAL COLONIZATION WITH *STAPHYLOCOCCUS AUREUS*

Nasal colonization prevalence

We recruited 756 participants at four study sites (Iquitos, Arequipa, Talara, and Lima). We conducted three visits: at baseline (October – November 2013), 6 months (April – August 2014), and 1 year (October - December 2014). The recruitment occurred at baseline (655 participants) and at the 6 months visit (101 additional participants). Our overall nasal colonization prevalence was 18.9% (n = 143), similar to that reported in similar study populations (58). We identified sex, site of recruitment, and respiratory diseases as risk factors for baseline nasal colonization, while an increased time of service (which was strongly correlated with age) had a small protective effect.

Baseline prevalence

Our overall baseline prevalence was 9.7%, (analysis of the first sample provided by 756 enrolled participants). This estimated prevalence was lower than previously reported in Peru and worldwide from studies in military and community settings. There were no previous studies in military populations in Peru or Latin America but we would expect the military to have a similar prevalence as the local community. There are only a few studies of nasal colonization in active duty military populations, primarily from the US and one from China. These other studies found that the prevalence of colonization in American recruits was 31% (24), while the Chinese military found a different rate depending if individuals were deployed to urban (24.6%) or suburban military centers (16.1%) (58). In both cases, the prevalence was higher than what we found at baseline. Only two previous studies were performed in community settings in Peru. One study included only children from Cajamarca and found a prevalence of 11.9% (14), while the second studied nasal colonization in a shantytown community of Lima, the capital city and found that among adults of different ages the prevalence of nasal colonization ranged from 20.4 to 39.6% (12). These two previous studies in Peru are comparable with others studies performed in Latin American countries that showed that the nasal colonization rates with *Staphylococcus aureus* in community settings was quite varied. In adult populations, the nasal colonization rates in Brazil range between 32.7% (56) to 40.8% (57); similar to the results found Colombia where medical students had a prevalence rate of 25% (4). The prevalence in the Peruvian military measured in this study was closer to the prevalence rate reported among adult students in Nigeria (14%) and healthcare workers in Nicaragua (6.7% to 11.6%) (11; 65). However, these studies were performed in community settings, schools or in hospitals,

and the populations were different from our study population, comprised only of active duty military personnel.

When we analyzed the characteristics of the participants and how they were associated with baseline nasal colonization, we found differences based on the site of recruitment, diagnosis of respiratory disease during the previous year, and use of dicloxacillin. Of the four sites, Lima is the capital city of Peru (with approximately 10 million inhabitants), while Arequipa (900,000 inhabitants) and Iquitos (420,000 inhabitants) are the most important urban centers in the highlands and the jungle. On the other hand, Talara is a small city compared with the other three (101,000 inhabitants). We found that Arequipa and Lima had the highest prevalence (14.0% and 11.3%, respectively), with Iquitos at 9.1% and Talara only 4.3%. Of these four urban centers; Lima, Arequipa and Iquitos had greater population size than Talara, and high population mobility due to travel, business, tourism and education. Although Talara is the most important port in the north of Peru, where oil refineries are located, the commercial movement is lower compared with the other three larger cities. These demographic differences may create differential access to common antibiotics that are still sold without a medical prescription at small pharmacies and drugstores. In addition, the distribution of antibiotics to each military health facility is based on the most prevalent diseases and the size of the base. The study sites in Lima, Arequipa and Iquitos include the administrative and operational regional offices, and therefore exceed the population assigned to Talara, which is only an operational base. The increased exposure to antibiotics in Lima, Iquitos and Arequipa could favor the development of nasal colonization with *Staphylococcus aureus* by eliminating

other commensal bacteria colonizing the human nares; unfortunately information regarding the most used antibiotics at each of the bases or local areas is currently unavailable.

We defined the risk factor of respiratory diseases as positive if a participant reported any of these conditions: asthma, chronic obstructive pulmonary disease or chronic bronchitis. These are chronic respiratory diseases, that can have acute exacerbations, with viral infections of the upper or lower respiratory tract, being the most common (36). A viral infection does not require antibiotic treatment, but in Peru the prescription of antibiotics for this condition is common practice. Especially concerning is the self-administration of this type of medication without a medical prescription. The exacerbation of respiratory diseases can lead to the prescription of antibiotics that can alter the normal microbiome of the nares and possibly eliminate competitors of *Staphylococcus aureus*, ultimately favoring the nasal colonization with this bacteria type.

The previous use of dicloxacillin was associated with baseline nasal colonization. Dicloxacillin is an antibiotic belonging to the beta lactam family, with good effectiveness against Gram positive bacteria like *Staphylococcus* that is used extensively for the treatment of skin and soft tissue infections. We had expected that the use of dicloxacillin might reduce the rates of nasal colonization with *Staphylococcus aureus*, but we found the opposite. Possibly, the normal microbiota in the nares is more susceptible than *Staphylococcus aureus* to dicloxacillin, therefore favoring the *Staphylococcus aureus* growth. Additionally it can be a marker of history of SSTIs. Another possible reason can be the misuse of the antibiotic. Dicloxacillin is prescribed usually for 7 to 14 days, but given the misuse of this antibiotic in the population, it is possible that the dose and time of prescription were not adequate because the participant did not complete the full treatment,

possibly not having sufficient exposure to the antibiotic for the elimination of *Staphylococcus aureus*. We were not able to collect information regarding the dose, time of prescription, where the antibiotics were purchased, or if the treatment was completed.

Nasal colonization during the follow-up

Regarding the change of nasal colonization during the follow-up, we observed an increase in the prevalence of nasal colonization over 6-12 months. Starting with a baseline prevalence of 9.8%, going up at 6 months to 12.4% and at 1 year to 20.4 %. This last measured prevalence was closer to those reported in Peru and other Latin American countries as explained before, as well as to the prevalence reported by Chinese researchers. This increase in the prevalence at each time of sampling is similar with the findings of Treesirichod et al., who found an increase in prevalence among Thai medical students (73). However, the change in nasal colonization is a dynamic process and prevalence increases and decreases over time as was observed in athletes where the changed appeared to be based on the season (18). This observed change in the prevalence of colonization might be due to the change of our study population along time.

The low rates could also be due to laboratory technical issues, related to the sampling technique, transportation or processing of the nasal swabs. The sampling procedure and the lab techniques were the same for each visit, but the recovery of more *Staphylococcus aureus* isolates may be related to the variability within the lots of nasal swab we used. We had two different lots of the same nasal swabs (BBL™ CultureSwab™); one lot was purchased by NAMRU-6 but due to the 2013 US Government shutdown, they could not be delivered on time for the beginning of the study (October – November 2013); therefore, we bought a second lot from two different local providers (recommended by

NAMRU-6) at the beginning of the study. Theoretically, the swabs from both lots should be identical; but we cannot be assured of that because we did not know how they were stored at the local providers' facilities and at the Peruvian custom's facilities (where NAMRU-6's lot was stored until it was delivered). These swabs should be stored at a range between 4°C - 25°C but we cannot be assured that those were the storage conditions before they were delivered to us. We used the first lot for the baseline visit, while for the 6 month and 1 year visit we used a mixture of these two lots; however we did not record the source lot for the swabs and therefore we are unable to assess if this was a reason for the difference in the rates found in the follow-up visits.

In addition, all our swabs had a rayon swab applicator, which is widely used for this type of studies; however, there is evidence that swabs with nylon flocked or cellular foam tips showed better recovery of *Staphylococcus aureus* than those with rayon tips; however this study was performed in an artificial nose under controlled lab conditions, so it is unclear whether this would have an effect under field conditions. (77) The sampling technique was the same as described by Ellis et al (23). However, it is possible that there were variations in the technique due to the movement of the head of the participant during the procedure. In some cases, after the initial introduction of the swab, the participants moved their head after expressing discomfort, which could affect the sampling process; this fact could have been corrected as the study personnel become more confident with the technique. Also, the available time slot for taking the samples was limited, and we were only able to take 1 swab from these participants (the swab procedure consisted of rubbing the tip of one nasal swab 3 times at each nare).

Another factor that could possibly explain the change in prevalence of colonization over time is how the samples were stored. We took the samples at specific locations inside each study base, far from the health facilities. At these places, we used a Styrofoam cooler box with ice packs to temporarily store the swabs after we took the samples, because once they have been used to collect a sample, they should be refrigerated immediately. Given that we used only one box, the continuous opening of the box to store the swabs could have altered the temperature, especially at sites with high ambient temperature (Iquitos and Talara), compared with colder cities like Arequipa and Lima. At the end of the sampling, these boxes were taken to the health facility at each study site and were stored in a refrigerator until the samples were shipped to the NAMRU-6 lab in Lima. The shipping of the samples usually took 1 day, but NAMRU-6 took longer than expected to process each group of samples, due to other competing projects. The first group of samples (at baseline) was processed after approximately 1 month, while follow-up at 6 months required 3 weeks and the one-year samples required only1 week. The samples were appropriately stored at NAMRU-6's lab. However, this different time for processing the samples for each sampling time may could have affected the recovery of *Staphylococcus aureus*.

These three findings can also help to explain the results of the reproducibility testing. We performed a reproducibility analysis, obtaining duplicate samples from 123 participants in order to assess the reliability of our sampling and lab procedures. The overall positive percent agreement was 0.68, which is far from a perfect agreement (1.0) to say that our methods are reliable enough to recover *Staphylococcus aureus*. When we analyzed them by operator, time of sampling and place of sampling, we found that the PPA was similar for two operators (0.6) but the third had a PPA of 1.0; the second sampling (6

months) had an overall low PPA (0.4) and also Iquitos and Talara had a PPA below 0.6. There were three operators trained in the sampling technique before the study started. One of them, with a PPA of 1.0, only went to two locations during the first two visits, before retiring from the Peruvian Air Force. The other two operators were present at all the sites during each time of sampling. It is possible that these two operators could have improved their techniques over time but this would have required further sampling to determine. Low agreement during the second sampling could be related with the different lots of nasal swabs that we used as we explained above. Talara and Iquitos have the highest temperatures, and the need to keep the swabs inside the single container we used while we were sampling may have affected the ability to recover *Staphylococcus aureus* samples.

There could be more variation than previous acknowledged in the sampling process. In those who were positive in the first sample and negative in the second, it is possible that we could have "cleaned" the nares after taking the first swab; while for those who were negative in the first sample and positive in the second, we could have missed them during the first swab due to variations in the sampling technique, movement of the participant during the sampling procedure, confidence of the operator for taking the first swab, etc. Therefore, the variation in the ability to obtain *Staphylococcus aureus* even if it is present in the nares can explain also the results of the reproducibility study.

These findings have implications for future research; including a previous standardization of all the sampling procedures before the start of the study, with proper training of the study team in the sampling techniques, adequate handling of the swabs before and after the sample was taken; proper maintenance of the storing devices, including the use of thermometers to check that an adequate range of temperature is maintained and

therefore the samples are adequately stored. In this way, we could reduce the potential pitfalls we have found with our reproducibility analysis.

Antimicrobial susceptibility and MRSA strains

We performed antimicrobial susceptibility tests on all the identified *Staphylococcus aureus* isolates (N = 183). Our results showed a remarkable susceptibility to different antibiotics currently in use in Peru, with rates of resistance below 4% for clindamycin, oxacillin, gentamicin and levofloxacin. The rate of resistance to erythromycin was 16.4%. Only two samples were identified as methicillin-resistant *Staphylococcus aureus* (MRSA) and therefore our overall prevalence of MRSA colonization during the study period was 0.3% (2 of 756). This prevalence was close to those reported in previous studies in Peru and Latin America, where it ranges from 0.6% to 1.8% (3; 12; 74) in communities; however these prevalences of MRSA are lower than those observed in developed countries like US or Europe. In terms of military populations, our results reflect that MRSA nasal colonization is lower than rates reported in US military populations (3%). The Chinese study did not detect any MRSA isolates and therefore we cannot compare our results (24; 58).

These two isolates were USA100, SCCmec type II, possibly related to the New York/Japan (NYJ) strain currently circulating in Latin America (29; 62). Both MRSA strains were collected during the second round of sampling (April – August 2014), but one belonged to a new enrollee, while the other was collected from a first round enrollee (October – November 2013) who was negative in the baseline sample. In Peru, 3 MRSA strains from Peruvian citizens returning from abroad were characterized in 2011, one was ST30 and the other two were ST8 clones which is related to the clone USA300 (27). To

date, this is the first time that this USA100 strain is reported in Peru. The New York/Japan strain was first isolated in Mexico in 1999, and later in Brazil in 2004, but it is distributed worldwide, being reported in US, UK, Europe, Saudi Arabia, Israel, Singapore, etc. (22). However, more molecular studies such as DNA sequencing are required in order to confirm these isolates as NY/Japan clones.

Risk factors associated with baseline nasal colonization

Multiple risk factors can affect the development of nasal colonization with *Staphylococcus aureus*, including genetic factors associated with the bacteria (adhesins), the host (genetic determinants associated with the immune system), competition with other bacteria in the nasal microbiome, non-modifiable factors including age, gender, geographic location; and modifiable factors such as use of antibiotics, previous exposure to healthcare settings, crowding living conditions, and smoking (67). In the case of nasal colonization with MRSA, the most significant risk factors described were the previous use of antibiotics, previous hospitalizations and previous diagnosis of skin and soft tissue infections (22).

In our study, we did not investigate genetic factors associated with the bacteria or the host. Instead, we focused on understanding the modifiable and non-modifiable risk factors associated with colonization among Peruvian military population. Our findings showed that being male, being deployed to Arequipa, Lima or Iquitos and having chronic respiratory diseases increased the risk of being colonized with *Staphylococcus aureus* colonization. Our findings are consistent with those described in the literature regarding the effect of sex (67). In terms of geographic location, being deployed to Arequipa increased the risk of having nasal colonization 4.5 times, almost doubling the prevalence of Iquitos and Lima. We have explained above how different factors related to these urban centers

could have explained why the prevalence increased compared to Talara; among these factors the size of population, differences in commercial activities, type of military facility, differential access to antibiotics, etc. may have been influential. Arequipa is the second largest city in Peru, with a population close to 1 million inhabitants, and our two MRSA isolates were isolated from this city. However, why this city has a higher risk requires further research; among the different reasons we should consider the great influx of tourists from differences parts of the world, who could be colonized and thus increase the chance of transmission to the local population, the climate, altitude, temperature, humidity (dryness), that could have affected the local microenvironment at the nose, favoring the nasal colonization with *Staphylococcus aureus*.

Chronic respiratory diseases are characterized by chronic inflammation of the airway, which can modify the physiological characteristics of the respiratory mucosae at the upper and lower respiratory tract. This fact can lead to a change in the local immune response, characterized by an increase in the presence of IL-8 in the nasal mucosa. IL-8 attracts neutrophils which keep an inflammatory response at this level (32). This inflammatory response can affect the "competitors" of *Staphylococcus aureus* at the nares, because this bacterium produces substances that can impair the phagocytosis and inhibit the recognition by neutrophils; thus overcoming a hostile environment in the nasal mucosa. We were unable to get information regarding the number of acute exacerbations of these respiratory diseases or regular use of medications, or any other physiological or immunological characteristic, but these should be the subject of further research.

The use of antibiotics increased the risk of being colonized by 40%, while the previous diagnosis of SSTIs reduced the risk of colonization by 60%. However these results

were not statistically significant. Being hospitalized in the previous year had no effect on the risk of being colonized. It is possible that the use of antibiotics increased the elimination of "competitors" for *Staphylococcus aureus*, and favored its settlement at the nares, as it was explained above; while to the contrary, the diagnosis of SSTIs could lead to the prescription of specific antibiotics whose primary target was *Staphylococcus aureus*. The Peruvian Air Force does not have a formal guideline for the treatment of SSTIs, but the usual first choice for antibiotic treatment of this diseases are dicloxacillin and cephalosporins of first and second generation, in accordance with international guidelines (1; 63). Therefore, while the other antibiotics could have favored the elimination of commensals at the nares, the treatment of SSTIs can effectively reduce the nasal colonization with *Staphylococcus aureus*. A physician usually diagnoses SSTIs and the participants would have been more likely to have had an adequate dose and completed the 7 to 14 days the treatment that was usually prescribed for these conditions. The Peruvian Air Force typically provided these medications free if they were available at the military pharmacy. Also, prior SSTI might have altered the host immune system to mount a response against all the *Staphylococcus* species, thus reducing the colonization with *Staphylococcus aureus*.

Change in nasal colonization over time

We analyzed the change in the nasal colonization status among those participants who provided at least 2 samples. Only 484 participants met this requirement (390 after 6 months and 94 after 1 year). The incidence rate of nasal colonization (those who changed from negative to positive status) was 11.2%, while the percent clearance (those who changed from positive to negative status) was 51%. These rates of incidence and clearance

were similar to the results found by Ellis et al. (8.4% and 50%, respectively) in similar populations in the U.S. (13). We found a statistically significant difference among the prevalence of nasal colonization at baseline and at the second sample. However, these results might have been affected by our losses to follow-up that reached 40% for this analysis. Our study population is subdivided in two subgroups: a permanent population comprised of the officers and Non-Commissioned officers, who pursue a military career, and a non-permanent population which correspond to the troops who usually serve for 1 or 2 years. The reasons for unavailability at each visit were different for each subgroup. The permanent population was often on temporal commissions outside the base, vacations or deployed to other military units in Peru and abroad; while the non-permanent population, the main reason for unavailability was the discharge from the military. Most of the troops voluntarily left the military service before 1 year of service. When we compared the characteristics between the participants that continued in the study and those who were lost to the follow-up, the statistically significant differences were rank (troops) and site of recruitment (Lima). However, the prevalence of baseline nasal colonization between these two groups was similar.

186 participants provided 3 samples and we were able to determine the status of nasal carriage over the full year in this group. Our prevalence rates are different from those previously reported in the literature (20; 36; 67). In all, 81.1% were never colonized 14.2% were occasional carriers, while 3.7% were intermittent carriers and only 1.1% were persistent carriers. Many of the longitudinal studies that assessed the carriage status were done with a small window of time, no more than 6 months, and the samples were taken every week, during variable time periods (18). In our case, the fact that we took the samples

after 6-12 months could have an effect on our patterns of *Staphylococcus aureus* colonization, indicating that over long periods of time, our participants may have cleared the colonization. However, due to the limited number of participants with three samples, we are underpowered to find any significant difference. Since our windows of sampling were 6 months apart individuals may have become colonized and cleared within that window without us detecting the colonization/clearance. To assess more frequent variation in colonization status would have required more frequent testing of our study participants.

Risk factors associated with change in nasal colonization status

We defined change in nasal colonization among the 484 participants who had two samples, and divided them in 3 categories: No change (84.5%), incident case (10.1%) and clearance of nasal colonization (5.4%). The "no change" individuals consisted of both colonized at both sampling times and not colonized at either sampling time. We analyzed in an exploratory way the factors associated with the colonization and clearance during the follow-up, using a multinomial logistic regression, comparing each change (acquisition and clearance) against no change and we found that in those participants with two samples, the diagnosis of skin and soft tissue infections during the time of follow-up, being deployed to Lima or Arequipa, or being mobilized to other bases increased the risk of being colonized in the second sample with *Staphylococcus aureus*. We conducted an analysis comparing both groups that changed colonization status to those that were non-colonized at both sampling times. The patterns of association were similar to those reported above.

We previously analyzed the effect of use of antibiotics and the geographic location on the risk of being colonized. Deployment of personnel is a common feature of military activity; the Peruvian Air Force has units dispersed throughout the Peruvian territory and

also carries out missions abroad, such as the peacekeeping missions in Haiti and Africa. These deployments may be of variable duration; but it could have increased the risk of acquisition of *Staphylococcus aureus* if the participants were deployed to areas with a high prevalence. Unfortunately we do not have information regarding the areas of potential transmission of *Staphylococcus aureus*, as most of the study participants were deployed or visited Lima during the follow-up time, and we could not get reliable information about the duration of the deployment.

The use of corticosteroids during the period of follow-up, being male and the place of recruitment were associated with an increased chance of clearance compared to those no change in colonization. However, none of the variables considered in this model were statistically significant. Use of corticosteroids reduces the inflammatory cascade, and could have altered the microenvironment of the nasal mucosa, which may have led to the clearance of *Staphylococcus aureus*. Our greater number of male participants may have artifact of the greater number of male participants, but since being male was recognized as a risk factor for colonization, it requires further research. The effect of site of recruitment was contradictory at Lima and Arequipa the risk of being colonized or cleared of infection exists simultaneously; it is probable that there are other variables that could have confounded this association, but we did not measure them.

Limitations

The main limitation of the study was that we could not reach the proposed sample size. This lack of sample size has an effect on our determination of risk factors, because we were then underpowered to detect a difference in their effect on nasal colonization. Another limitation is the loss of participants over time due to discharge, retirement, and military

activities outside the base at the moment of the visit. These could not be anticipated beforehand; we expected that some personnel would retire by the end of each year, and not at any time during the year. A similar situation developed with the troops who left the service after only 6 months – again, not expected. Definitely, these events should be addressed in future research, possibly by reducing the study interval period, or by restricting the study population in order to minimize the losses to follow-up, etc. When we compared those who contributed 2 samples vs. those who were lost to follow-up, we found statistically significant differences in terms of rank, place of recruitment and use of antibiotics. We lost more enlisted troops than other ranks probably due to the rapid turnover of these personnel. The troops constituted a non-permanent population who usually serve only 2 years. We lost 43.8% of all the troops initially enrolled in the study, and this could have had a differential effect on our estimation of risk factors because of their activities. Further, troops have a greater risk of having wounds or infections that may require the use of antibiotics or other medication because of their occupational exposures and closer living conditions.

Our losses to follow-up were highest in Lima, where we did not have the opportunity to recruit more participants. At this base, some of the enrollees declined further participation arguing that the nasal swab was a test for illegal drugs use; despite our efforts to explain this error of perception, they decided not to continue participating in the study. Also, we had to postpone our visit twice at each sampling time due to other military activities that were mandatory for this base (like the Military Parade for National Holidays). This unavailability of personnel in this base could have affected our estimates of nasal colonization, and artificially increased the rates at Arequipa. Those who were lost to

follow-up were less likely to report the previous use of antibiotics, and this could also affect our estimate of risk, decreasing it artificially.

Our questionnaire was simple and designed to be completed in less than 10 minutes, given the small window of time we had at each base for the execution of the study. Therefore, it was not designed to provide more specific information regarding the type of SSTI and treatments prescribed, dose and timing of antibiotics and corticosteroids, time of hospitalization, place, and antibiotics used during this time, which could have given us more detailed information regarding these risk factors. Also, given that this was a self-administered questionnaire, there is the potential for recall bias. In addition, it is possible that some participants did not understand the questions asked or did not know the terms used; therefore they left the questions blank.

Another limitation of this study is related to our sampling and lab procedures, given that our reproducibility analysis shows that the reliability was not high. Our sub-analysis showed that two operators had a similar agreement, but those samples taken during the second collection, and those at Iquitos and Talara had the lowest agreement. This could be explained perhaps due to the use of different lots of nasal swabs at that time, the temperature and shipping conditions as previously described, or variation in assessment of *Staphylococcus aureus* colonization not previously acknowledged. In addition, we were unable to assess the effect of those who processed the samples at the lab, because no list of laboratory technicians was available.

Challenges

We faced several challenges in the execution of this study. It took us two years to organize the study and get the approvals from the Peruvian Air Force, USUHS and

NAMRU-6's IRBs. Once the study was approved, we were delayed due to the US Government shutdown that led to the use of two lots of swabs instead of one for the entire study. There were additional issues with base access by a civilian academic (PI) as well as lack of promised personnel support due to unforeseen missions.

The fact that the personnel were deployed to a particular base did not mean that they would stay there during the full period of deployment, unlike other military forces around the world. There was a high level of mobility within the Peruvian Air Force during our study, many times without previous notice. These movements were mainly to operational bases (combat) or Special Forces units. Additionally, retirement occurred at any time as well as unplanned discharges (especially among the troops). Another challenge we faced was related to logistical issues. We were only allowed to conduct the study during the first few hours of the day (8:00 – 9:30 am). If individuals missed this time slot, they usually did not return to the study site to be included in the scheduled sampling, and were thus lost to follow-up.

Another challenge that took time to overcome was related to the NAMRU-6 lab and the variable time to process the samples (from 1 month for the first group of samples to 1 week for the third group). Despite previous coordination, we had to use the first day (Monday) of our 5 day visit to inform the highest ranking officer of our activities, and later we had to coordinate the authorizations for the place of sampling, support personnel and storage of the swabs. We only had three days to complete all the sampling in order to send the shipping package on the fourth day (Thursday) so as to arrive at NAMRU-6 on Friday before the weekend. The cold conditions inside the shipping package may have deteriorated after 1 day. These conditions are typical for the developing setting and are not meant as

excuses, but do represent the real work conditions of performing research in Peru and likely other lesser-resourced areas.

The greatest challenge was related to the change of authorities in command of the Directorate of Health of the Peruvian Air Force. It usually occurs every two years, but in 2014 there were two changes in command, one in June 2014, and the other in December 2014. These changes generated issues with the execution of the study because a new agreement with each Director was required and negotiation ensued in order to ensure the continuous support for our activities. This change in personnel affected the authorization for travel of the study personnel. Despite initial support, there were several suspensions of our activities due to the denial of travel authorizations. We faced this situation before we could take samples at Lima, which contributed to the highest loss to follow-up at this base.

Public health implications

Our results increased the current knowledge about *Staphylococcus aureus* nasal colonization in Peru and Latin America. As indicated above, few population-based studies have been performed in the region, most of them in populations associated with healthcare settings; and there were none previously in military populations. This is the first study that assessed simultaneously the prevalence of nasal colonization with *Staphylococcus aureus* in four different cities of Peru among active duty military We believe our results can serve as a proxy to understanding of the patterns of nasal colonization with *Staphylococcus aureus* in the community. In addition, we found that these isolates have a remarkable antimicrobial susceptibility with very little resistance when compared with other populations. This susceptibility allows the standardization of the treatment of different infections where *Staphylococcus aureus* is a common etiologic agent, following international guidelines

from PAHO. This standardization should help to reduce the indiscriminate exposure to more expensive and broader spectrum antibiotics that should be left as second or third line options, which are more expensive and increase the risk of adverse reactions.

We identified the deployment of military personnel as a risk factor for increasing the change of nasal colonization. This fact has serious implications for the transmission of *Staphylococcus aureus* not only among military personnel but also among their contacts in the community. This exposure could change the rates of nasal colonization, and increase the circulation of strains, including the new MRSA strain that we identified as circulating in Peru. The knowledge of most of MRSA strains identified in Latin America comes from the current laboratory surveillance network sponsored by PAHO that relies on samples identified at tertiary-level hospitals in urban centers at different Latin America countries. We analyzed data for the 2013 WHO Global Health report on antimicrobial resistance using the datasets from this surveillance program. PAHO has expressed interest in our results as these results come directly from the community, a feature absent in the current PAHO surveillance network.

A clear gap in the public health study of MRSA and other antimicrobial resistant bacteria is the lack of a repository that can relate strains to clinical outcomes and molecular findings. This sample repository could receive samples taken from the community as well as hospital and allow the public health and academic communities to perform molecular epidemiology studies. Findings form these studies would help Peru design better strategies to prevent and control resistant strains, and increase our understanding of genetic, demographic, and behavioral factors that may inform these strategies.

OBJECTIVE 2: SKIN AND SOFT TISSUE INFECTION SURVEILLANCE

SSTI prevalence

Skin and soft tissue infections comprise a broad number of clinical conditions, characterized by the infection of one or multiple layers of the skin. These events can become life threatening if left unattended. In military settings, they are an important cause of loss of working days and affect the readiness of the military personnel and ultimately military operations. The current status of surveillance in the Peruvian military still relies on paper log-books, but the data does not arrive to the Directorate of Health in a timely fashion. It is important that this paper-based system be replaced with a more accurate and timely system.

Our findings show that the annual rates of SSTIs were below 10% for each year assessed (2012 – to 2014), with cellulitis being the most common diagnosis (52.2%). Apparently most of these occurred during the first quarter of the year (coincidental with the summer) consistent with other regions (2); SSTIs affect more non-commissioned officers (NCOs) than the enlisted troops unlike reports from other parts of the world (44; 45). We could not assess the number of lost working days, nor the treatment provided for many of the registered cases, because these data were not collected in most of the health facilities.

Our prevalence estimates of SSTIs varied by year, showing a decrease in the number of cases of SSTIs over time (from 2012 to 2013). In 2014 there was also a reduction but this can be an artifact because information form the two largest hospitals at Lima could not be included in this analysis and it would e difficult to compare with previous years. We could not get information from the two largest hospitals in Lima for 2014, which may explain the drop in the SSTI prevalence for that specific year. Another

possible explanation is related to the availability of infectious disease physicians or dermatologists who can make a more accurate diagnosis of some of these conditions. Currently, the Peruvian Air Force is facing a shortage of physicians and qualified health personnel.

Our prevalence rates follow similar patterns over time, but there are regional differences; for example in the southern region there are two peaks in the number of cases, one in April and the other in October, while in Lima, the main peak occurs in February and then the number of cases declines over the rest of the year. It is also probable that the observed peaks may be affected by the entry of new personnel as cadets, NCOs in training and troops. These new personnel undergo basic training, which is demanding and can increase the risk of skin and soft tissue infections.

Another striking finding is the lack of information regarding the antibiotics prescribed for the SSTIs treatment. Slightly more than 7% had the prescribed treatment in the log-books, and the treatments varied. We could not collect information regarding the dose and timing of the prescription in each case where the treatment was recorded, but the antibiotics used included beta lactam derivatives, cephalosporins, aminoglycosides, fluoroquinolones, and macrolides. Many of these antibiotics are second-line treatments and in some cases they are the optional treatment in case there are allergies, or the strain is related to healthcare settings. The broad availability of these treatments may increase the antimicrobial resistance, thus rendering previously effective antibiotics useless, ultimately increasing health costs.

Evaluation of the skin and soft tissue infection surveillance system

In terms of the skin and soft tissue infection (SSTI) surveillance system, the addition of SSTI conditions to the system was implemented but its performance after 9 months of functioning was below our expectations. Only 2.2% of all the SSTI cases diagnosed during the period April – December 2014 were reported in the system; and of those included in the system, only one matched the record in the log-books. Therefore, our sensitivity was very low. However, the timeliness was 100% for the 6 cases that were reported into the system. This poor performance can be attributed to several factors. The scope of our evaluation was only to assess the usefulness and the performance of the system measuring some attributes, using the specific CDC guidelines for measuring them. The task of evaluating a surveillance system is difficult, because it involves the collection of information from multiple sources, including all actors involved in the processes of the system, and willingness to be evaluated, a feature that is difficult to overcome in developing settings like Peru, especially when after more than 10 years of functioning, the current electronic surveillance system has not been fully evaluated. This lack of a formal evaluation of the system is not unique however, given that there are only a handful of published evaluations in the literature (8; 9).

Structure of the system

According to the WHO framework for evaluating epidemiologic surveillance systems, there are four areas that must be evaluated: structure, core functions, surveillance quality, and support functions (10; 28). In terms of structure, the SSTI surveillance is part of the infectious disease surveillance system of the Peruvian Air Force. Its inclusion in the system was required in September 2013. The reason for the inclusion came from

information collected by a research project executed at the Central Hospital of the Peruvian Air Force, where samples from surgical wounds are collected and sent to NAMRU-6 for isolation and antimicrobial susceptibility of the pathogen. Some of these isolates were MRSA and prompted the concern of the health authorities for the presence of MRSA in the active duty population. Given that skin and soft tissue infections are the most common infection associated with *Staphylococcus aureus*, the Peruvian Air Force asked for its inclusion as a separate event in the surveillance system because there were reports of SSTIs included as "Others" in the existing surveillance system. The main objective of the addition to the surveillance system was to assess baseline rates of SSTIs in active military population

This system works under the Health Directive 160-14 issued by the Directorate of Health of the Peruvian Air Force with mandatory execution for all the health facilities of this branch. The system was designed using free software (Linux) and the Bio Informatics Unit of NAMRU-6 performs the technical maintenance activities without cost to the Peruvian Air Force. As it was explained in the methods, the reports are sent by phone and Internet, and they appear immediately in the main webpage of the system.

Core functions of the system

The core functions of a surveillance system are case collection, analysis and dissemination of the information. The system is designed to collect baseline information for almost 50 infectious diseases, and detect outbreaks in real-time. It receives information from reporters (stakeholders) by phone or Internet, and the information is immediately updated in the dataset of the surveillance system. Once the information is collected, this is analyzed by the administrator of the system (one for the Peruvian Air Force), who detects

errors, monitors the report on time, and also prepares weekly reports. The case collection is directed by the reporter and depends heavily on the registration of the case in the log-books or other instruments. As we described above, there are several issues involving the sources of information that can affect this activity. Another important factor is that the reporters are trained to send two types of events: Collective reports (that are sent on a weekly base) and Immediate reports (that are sent as soon as a case appears). During the training sessions, more importance is given to the collective reports because these reports occur on a scheduled interval and there is a deadline for sending the information. On the other hand, the immediate report is performed when the case occurs, and given that they are not frequent events, it is likely that the reporter is more concerned with sending the collective report, usually the indicator for evaluating the report on time of the system. During our evaluation, many reporters suggested to the team that SSTI cases should be sent on a weekly base instead of at each time a new case appeared after we showed them the poor performance of the system; apparently this "perceived" importance of the collective report might help to explain the low rate of report of SSTI cases.

The last activity related to the core functions is the dissemination of the results, which the health authorities must conduct. As mentioned above, despite the enthusiastic initial support, this waned over time, not only with the SSTI surveillance but also with the whole surveillance system. The administrator prepares official reports each week, and they are sent each trimester to the Directorate of Health, but these reports are never published and therefore, the health personnel do not have information regarding the use that the Force gives to the data they collect and report. This behavior undermines the "perceived" importance of the activities of the health personnel, and may lead them to question the

value of executing these tasks. Also, this can affect the commitment of the local military authorities to encourage the surveillance activities, because they do not see any consequences as result of the surveillance activities.

Usefulness and attributes of the surveillance system

We used the 2001 CDC framework to evaluate the SSTI surveillance system in order to assess the surveillance quality through the assessment of the usefulness and the attributes of the SSTI surveillance system. This framework suggests the measurement of the following attributes: simplicity, flexibility, data quality, acceptability, representativeness, timeliness and stability (28). Simplicity is based on the structure of the system and how easy it is to send a report. This system required the collection of cases that matched SSTIs diagnosis or relevant ICD-10 codes; therefore, the reporter was not responsible for making the diagnosis of each clinical case; once the information was collected from the log-books, it had to be entered into the on-line system, completing a questionnaire by phone or Internet. Our questionnaire had 20 questions, but only 7 of them were mandatory. The average time to enter the report calculated during the training sessions by Internet was approximately 3 minutes per report. The information was available immediately for the administrator located in Lima who could monitor the system in real time, checking for errors or duplications in the report. Based on these measurements, our system is simple in structure and simple to use; however, our system was underused because we only received 2.2% of all the SSTI cases diagnosed during the period April – December 2014.

In terms of flexibility, there were no requirements for a modification in the questionnaire of the SSTI surveillance system, so this feature was difficult to assess.

Reporting SSTIs by phone was not accomplished however, which limited the ability to use the system and thus was not flexible. As it was explained in our results, this was due to logistic and technical issues at the Bio Informatics Unit of NAMRU-6; and implies that new requirements would be dependent on human resources and equipment available by NAMRU-6, compromising the flexibility of the system. Unfortunately there is still no technological transference between NAMRU-6 and the Peruvian Air Force, and this is a setback because system remains dependent on time and resources from NAMRU-6. To date, the system cannot be managed by the Peruvian Air Force due to the lack of funding and resources, possibly explaining the reluctance of the Peruvian Air Force to provide additional support to the use and implementation of the system.

Data quality could only be assessed partially. However, the timeliness for the few reported cases was 100%, but the sensitivity for case detection was low, less than 5%. Our data quality measured by completeness of report and error rate, could only be measured in one report that had a match in the log-book, and it was complete and without errors. These characteristics are addressed during the training sessions, but in our case these training session took place 6 months before the start of the SSTI surveillance program. Despite the fact that we left them the training material, and asked the administrator to provide additional online training or on-site training at each visit available, additional training and turnover training was not carried out and certainly affected the performance. The acceptability is a measurement of the participation and willingness of each participant of the system to execute the surveillance activities. In our case, the three main actors: authorities, administrators and reporters showed inconsistent acceptability. In the case of the authorities their support and encouragement of the SSTI surveillance system waned

over time, mainly due to the changes in the command and the lack of vision regarding the importance and opportunities that the system could offer to the Institution. This attitude also affected the broader electronic disease surveillance system. The administrators did not have the appropriate medium (phones) to execute their activities. In the case of the reporters, behavioral (perception of duplication of activities) and logistic issues like lack of reporting tools, or execution of other military tasks with high priority affected the acceptability of the system. During the informal interviews we had with them, they expressed their willingness to use the surveillance system. The representativeness of the system, which refers to the accuracy of the information collected about the event under surveillance over time and the ability to represent the population under surveillance, was limited. In our case, with only 1 case, we are not able to assess this attribute.

Support functions of the surveillance system

Finally, the support functions also played a role in the poor results of our surveillance system. Among these are training activities, supervision and resources. We already discussed the effect of the lack of resources for sending the report, as well as the specific training activities for SSTI surveillance. In terms of supervision, there is a designated administrator of the system per each branch of the Peruvian Armed Forces who monitors the activities of the infectious disease surveillance system are in charge of the administrator of the system. They consist of the assessment of data quality and report on time, organization of training activities, and monitoring of the functioning of the system. This person was usually stationed at NAMRU-6 in Lima, where all the technical equipment was available for monitoring, including phone line, personal computer and Internet connection. A critical activity is monitoring the report on time, which usually consists of

calling the stakeholder early on the deadline day and giving him/her a reminder for reporting, which is the most effective way to improve the report on time as it is reported in the literature (31). Unfortunately, after the first change in command, the presence of the administrator at NAMRU-6 was reduced to only 1 day per week, and therefore, the monitoring activities were restricted. At the same time, the training activities were reduced due to budget issues and instead of being done twice per year (including in this way to the new health personnel that was mobilized in June for the rural service), occurred only once a year for 2013 and 2014. Therefore, the new personnel did not have the appropriate training for sending a report, a reality we faced during our last visit to each health facility.

This surveillance system had poor performance during the period of evaluation (April – December 2014). It was not useful for the collection of the SSTI cases. It was however simple to use, collected all the information required, was accepted partially by all the personnel involved. In the end, however, the system was not used. The timeliness evaluation was limited to only one case, and we could not assess whether it was representative of the health of the population. Our findings show that there are still many challenges to perform in order to improve the performance of this surveillance system. Unfortunately we cannot compare our results with previous reports of evaluation of infectious diseases in the literature due to our lack of data. However, the literature also includes reports with many challenges, including in developed countries like the US; ESSENCE II showed excellent performance and excels in their attributes (41), but Armenia and Japan reported that their systems were inefficient and costly (81), or did not receive information on time or had quality issues (43).

Limitations

There were a number of limitations in this component of the project. Disparity existed in our sources of information for the collection of SSTI cases. The Peruvian Air Force issued a Directive mandating the use of numbered log-books to register all types of provided medical care, but we only found them used in 2 of the 27 health facilities; the remaining 25 bases used a variety of registration forms which included, informal log-books, datasets, or even nothing. When we could not find a log-book or dataset for a given period of time, we reviewed the prescription receipts or the daily record sheets in order to collect the cases at some of the bases.

Unfortunately these alternative data sources were not as complete as a definitive as an official log-book. Also, there were bases where we could not get information for the whole year, because the inexistence of records for some periods of time, which were coincidental with the absence of a physician or health personnel in the base, the destruction of the log-books due to lack of storage space or even natural disasters (floods). This occurred especially at some bases outside Lima. Also, at regional hospitals, there is often more than one log-book, and we could only review those corresponding to outpatient and emergency services, but not those from inpatient services.

Another limitation regarding our prevalence estimates is that we were very strict in our definitions of skin and soft tissue infections. All cases included were those which were written in the log-book, and that we recognize as an SSTI. In the event the ICD-10 code was the only indication of diagnosis, we only collected those corresponding to L01, L02, L03, L04, L08 and L73. However, it is possible there could have been a mismatch between the diagnosis and the ICD-10 code when they appeared together, because completing the

log-books is not an activity performed by the physician, but by other health personnel. Only the physician should complete the daily record sheet, but usually they only fill the diagnosis and leave the ICD-10 code to be filed up by another person; therefore, this other person must look for the ICD-10 code and will not necessarily be accurate in the written code. These factors could have led to a greater underestimation of our prevalence estimates. This underestimation was specifically an issue for 2014 because we could not get information from the two main hospitals in Lima, which have a great influx of outpatients and inpatients.

Challenges

In previous research we identified the challenges that rise in resource-limited settings when a surveillance system is implemented (68).(68) Unfortunately, after 6 years they remain unchanged. We expected a quick and untroubled implementation of the SSTI surveillance system, given that the infectious disease electronic surveillance system has been functioning for four years, and the Peruvian Air Force possesses fewer health facilities compared with the Peruvian Army and Navy, which are counted in hundreds. In addition, the system receives collective reports on a weekly basis and before the implementation of the SSTI questionnaire it was evident that a "surveillance" culture existed among all the personnel involved in the surveillance process. Despite all these facts and expectations, the SSTI surveillance system performed poorly.

The first challenge is related to political issues. The incorporation of the Peruvian Air Force in the electronic surveillance system occurred in 2010, after the formal request from the Directorate of Health. Since then, this Directorate deployed a non-commissioned officer to NAMRU-6 to monitor the performance of the system, coordinate the training of

the stakeholders and to create quarterly reports. This system continued until 2013. The Directorate of Health of the Peruvian Air Force recognized our surveillance system as important. However this enthusiasm did not continue over time. Unfortunately, during 2014, there were two changes of Commanding Officers at the Directorate of Health; and at each time, we had to explain the activities related to surveillance, but despite an initial vow for collaboration, it was not translated into action. This lack of political support from the higher ranking authorities may have influenced the situation increasing the problems we faced with the SSTI surveillance.

The Directorate of Health issued Directives related to epidemiologic surveillance, but they have a general scope and do not mention specifically the mandatory use of the electronic surveillance system. As it was explained before, the main source of health data for the Peruvian Air Force is paper-based log-books and they are used to collect information for the reports that the Peruvian Air Force must send to the Peruvian Ministry of Defense on a quarter base, and also for the local Regional Directions of the Peruvian Ministry of Health. Therefore, they already execute surveillance activities, and sending the report into the surveillance system represented a duplication of activities, since they already reported via the paper-based surveillance system in compliance with the Directive about epidemiological surveillance. This perceived duplication and lack of reinforcement of the electronic surveillance activities might play a role in the performance.

Other evidence of this neglect is the underutilization of the system capabilities. The electronic surveillance system not only receives reports, it also has an outbreak detection module, a training module with video tutorials in the webpage, and it allows the export of the information as an Excel spreadsheet in order to facilitate the analysis by any person

with access to the system. Despite all these advantages, the Directorate of Health still trusted the paper-based records that are entered in Excel datasets by the health personnel at each heath facility. When it was implemented, one of the objectives was that over time, this electronic surveillance system may replace the current paper-based system; but this never occurred. Unfortunately this lack of concern is also transmitted to the lower levels, where the perception of duplication of surveillance activities and the perception of doing a "useless" job was observed by the study personnel. Definitely these beliefs could have affected the functioning of the SSTI system.

In terms of personnel, our main challenge occurred at two levels: administrators and reporters. We faced two changes in the command during 2014, as was explained above, and each change generated a reduction in the number of the days that the Peruvian Air Force system administrator was deployed at NAMRU-6. The first change took place on June 2014, and we observed a continuous decline in the report of SSTIs since then. The administrators of the system had to dedicate more time to the other activities of the Directorate of Health, and one of them retired from the Peruvian Air Force by September 2014. The other administrator, who was stationed at NAMRU-6, was responsible for monitoring the system, but these activities were reduced after the first change of command. Unfortunately, at the Directorate of Health, this administrator did not have any tools/equipment to effectively monitor the system, including no phone line or Internet connection. At least a phone line could have allowed a continuous monitoring, because this action was identified as the best way to improve the reporting to the system in previous research (31). In addition, given that he was the only person responsible for the Epidemiology area, he had to assume other functions previously performed by the other

administrator. The second level occurred with the reporters, because we had a high mobility of the stakeholders, and therefore, new personnel arrived to some of the health facilities without formal training in reporting to the system. Also, these new personnel were also responsible for other military activities and lacked time to send the report, as they expressed during our visits. These two facts impaired the adequate functioning of the SSTI surveillance system.

Another challenge was the lack of reporting media by the stakeholders at the health facilities. More than 80% of the health units sent the report by phone; but unfortunately, the SSTI surveillance was available only by Internet, due to technical difficulty with the development of the questionnaire as a voice record to be included in the report by phone. Also the report by Internet requires an electronic device like notebooks or tablets, and a functioning Internet connection, in contrast with the report by phone that only requires a functioning device. In this way, the reporters were burdened with finding an Internet connection outside the health facility, often using their personal computers and adding an extra activity to those they performed outside the military base. At locations where a computer was available, the Internet connection was often nonexistent for the health facility, also worsening the performance of the system.

In terms of support activities to the SSTI system, our main setback was the lack of scheduled training during 2014 in contrast with previous years. During the evaluation visit, we met stakeholders who were supposed to report to the system since they arrived at the health facility but could not do it because they did not have a formal training. This training is a two-day workshop where there were lectures and practices about how to report the different diseases under surveillance, and at this time they received training material and

also their username and password. One of the recommendations to maintain high levels of reporting was the training of their replacements at each site in order to avoid epidemiologic silences during the change of personnel. Unfortunately, this recommendation was not followed at any of the health facilities where the change of personnel occurred.

Public Health implications

Epidemiological surveillance is one of the fundamental activities of Public Health. The results of this surveillance are useful for the knowledge of any health event, the design of preventive and control measures, and generation of hypotheses. The Peruvian Air Force has executed this activity since the creation of the Directorate of Health, but it was not until recent times that they could use a system that provides information in real time. However, the lack of willingness from the authorities, the lack of monitoring activities, and the underuse of the capabilities of the system made it not very useful. This is a lesson for health programmers and Public Health practitioners in Peru and elsewhere, because once the surveillance activity fails, it will affect the future development of strategies and research because without reliable data those processes are impossible. Moreover in developing settings, where indicators of success of health programs and interventions rely heavily on accurate data, a functioning surveillance system is needed.

We identified several challenges that need to be addressed in order to optimize the surveillance activities. Regarding the personnel, it is important that all those involved in the surveillance activities interact with each other in order to know all activities and create an integration of efforts. This effort should become health policy for the Peruvian Air Force, instead of another collaboration with a foreign institution, capable of sustaining the activities over time.

Regarding the resources, it is important that the system can facilitate the activities of the reporters, providing them with all the necessary reporting tools. In this situation, it is important that reporting by phone be possible since the majority of sites do not have hard-wired Internet connections. In addition, the administrators should have appropriate equipment available to perform their activities,

CHAPTER 5: Recommendations

As in many research projects, our study has answered some questions but has identified several other important areas that should be addressed in the future, including further studies on the epidemiology, surveillance, clinical characteristics, host genetics, and microbiology of *Staphylococcus aureus*. Despite multiple challenges during this study, our findings have contributed to the base of knowledge concerning *Staphylococcus aureus* nasal carriage in Latin America. It has also generated numerous follow-on questions that should be addressed as well as many lessons learned. Below discussion involves recommendations based on the five core areas of Public Health (Health Policy and Administration, Epidemiology, Biostatistics, Behavioral and Social Sciences, and Environmental Health). These are organized as general statements and then I give some topics for future research in each of these areas. .

A. HEALTH POLICY AND ADMINISTRATION

It should be desirable that the Peruvian Air Force can implement a policy of biomedical research that can be sustainable over time and that can avoid the effect of the change in the line of command. Our activities were suspended twice due to the change in the "willingness" to collaborate with the research efforts. This policy would set a precedent that can be followed by the other branches of the Peruvian Armed Forces. Under this policy, training activities to improve the capabilities of the personnel at the Directorate of Health can be designed, and therefore research could be promoted. Instead of being an individual effort where the Peruvian Air Force only provides the participants, the Peruvian

Air Force would become an active player in order to get information regarding the current health status of its population.

The research efforts must continue, especially in military populations where so much is unknown related to health events. This study included the two members of the Peruvian Air Force who were part of the study team. At each study site, more people were involved and showed genuine interest in the execution of the study. Some research ideas not related with the study developed during our visits to each health facility, not only by the health personnel, but also the command. Many of these research ideas related to occupational health or chronic diseases. An added importance of our research effort was that we showed the Peruvian Air Force personnel that they were able to fully execute a research study. Unfortunately this interest was not necessarily matched at the Directorate of Health. To institute a research policy would create an opportunity to empower the Peruvian Air Force as a leader in research and set an example to the other two branches of the Peruvian Armed Forces. Some of the areas that require further research are:

1. How to maintain a sustainable surveillance system must be addressed. The SSTI surveillance system was implemented under the assumption that the conditions of activity and monitoring of the system would be similar to those of previous years. However, we found that changes in political willingness definitely affected the performance of the system. Possible scenario analysis, impact evaluations and risk management should be performed.

2. Comprehensive health information should be consolidated. Health information of the active duty military population should be unified. Epidemiologic and pharmacy information (specifically about antibiotics consumption) should be shared and managed by only one Department. The epidemiological characteristics could be linked with the therapeutic effect, improving the clinical management as well as preventing the development of antibiotic resistance.

3. Characterize the relative use of different antibiotics and resultant microbiologic and economic consequences. Economic analyses are needed in order to make decisions that can favor the health initiatives of the Peruvian Air Force. The determination of the different antibiotics and schemes used for the treatment of SSTIs can help us to calculate the costs associated with the medical care. However, these economic analyses can have a long temporal horizon in this study in order to determine accurately real costs and the effectiveness of different interventions.

B. EPIDEMIOLOGY AND BIOSTATISTICS

Epidemiologic Research

Antimicrobial resistance among bacteria such as *Staphylococcus aureus* is an underdeveloped research area in Peru. Most studies are limited to the report of identified strains at healthcare settings, and there are few studies providing information regarding the preferences of health practitioners and patients about the use of antibiotics. Given the increasing threat of antimicrobial resistance worldwide, studies at the community level should be performed and the information gained applied to the clinical setting. Information about the knowledge, practices and attitudes of all the stakeholders should be investigated

in order to design proper measures that can counteract antimicrobial resistance through education, adequate use of antibiotics, adequate prescription practices, availability of antibiotics based on regional profiles, implementation of antimicrobial stewardship measures, etc. Further research is needed to fill the current gaps in knowledge:

1. It is important to perform studies in different areas of Peru and South America, at the urban and rural level, in order to get a clearer picture of the current situation. Nasal colonization with *Staphylococcus aureus* in Peru does not appear to be as widespread as in other settings

2. Studies with a short interval between samples must be conducted to assess accurately the nasal carriage and if there is a change in the nasal colonization status over time.

3. Inclusion of immunological factors and other non-modifiable risk factors for nasal colonization with *Staphylococcus aureus* in future studies is warranted. We also must investigate the effect of other chronic diseases, specifically skin diseases like atopic dermatitis and psoriasis that are associated with high rates of colonization.

4. It is important to have a profile of the most used antibiotics at each urban center. Current information in Peru is limited only to some hospital settings, but not at the community level, where the access to antibiotics without medical prescription and the misuse of them is more frequent.

5. Further studies must focus on the impact of nasal colonization on prevalence of skin and soft tissue infections. Previous research has shown its impact on the rates of SSTIs specifically in military personnel in the US.

Epidemiologic Surveillance

In terms of epidemiological surveillance, the use of all the capabilities of the current electronic surveillance system must be promoted. This should start with an improvement in the communication between NAMRU-6 and the Peruvian Air Force whose collaboration started in 2009, specifically with the implementation of the electronic surveillance system. The health authorities should understand the importance of the system and how it can improve their current surveillance activities, complementing and not duplicating their efforts. The great advantage of the electronic surveillance system is that the information will arrive in real time. However the lack of interest in this timely information could be a result of the lack of an appreciation of its potential value. We propose the following research topics:

1. Determination of the best way to report an event with a low rate needs to be studied. The most reported events are those with a higher rate by week, like acute respiratory infections and acute diarrheal diseases, but they require less information than the report for other diseases. The questionnaire might be simplified in order to increase the reporting rate; but we must know exactly what information the Directorate of Health requires.

2. We must investigate the impact of the inclusion of alternative media to access the reporting system, including smartphones, which can allow reporting by phone or Internet. Previous research shows that smartphones are a good alternative, especially in Peru (and actually many developing countries) where the presence of these devices is broad and covers the entire territory.

3. It is necessary to identify which factors can modify the lack of interest among the stakeholders and measure their impact; we should align the activities of the system with the baseline activities of the personnel responsible for the health activities at each facility. This process may include the development of data analysis using Excel, in order to show them the utility of the data that they can obtain through the surveillance system. Also, the careful and appropriate use of incentives should be explored for improving the surveillance system's performance; these could be extra payments, or other novel initiatives such as public recognitions, enrollment in training specific for this task, etc. However, which of these incentives will be the best is matter of future research.

4. Economic analyses are needed that can assess which is the most cost-effective method of reporting, as well as the associated costs of the implementation and modification of existing surveillance systems. This analysis might also include the costs for the Peruvian Air Force to determine if the Institution will be capable of managing the system without external support. In the same line, this could be an opportunity for the

Peruvian military to save money across the health care spectrum, and to develop expertise that they can provide to other institutions within the Peruvian government.

Laboratory capabilities

All lab materials should be secured in advance, ready for use and properly stored before the study activities start. Likewise, the samples should be processed as soon as they arrive at the respective laboratory to minimize potential effects on culture yield. It is necessary that the Peruvian Air Force play an active role in the development of the study activities, recognizing that it will benefit the institution and it is not merely an addition to the usual work. For further research, that each study site must have the proper equipment for the storage of materials and samples. Examples of further research are:

1. Design and implementation of a specimen repository for resistant samples where molecular techniques can be used to identify not only the strains, but also genomic sequences and link them to clinical and epidemiologic risk factors

2. Mobile labs should be designed and tested to allow the rapid processing of samples without the need to ship to labs distant from the study sites. These labs would allow the isolation *in situ*, and then the isolates could be frozen/stored and shipped to reference labs for further study, improving the overall performance of our lab methods to recover the pathogen. Mobile technology could be used to share the information gained in real-time with reference labs.

3. Microbiome studies and examination of biofilms must be conducted given that the nasal colonization with *Staphylococcus aureus* depends heavily on interactions with other bacteria present at the nares. In this way, we will be able to assess the interactions between the simultaneous colonizers of the nares and how these change over time.

Quality control assessment

Our reproducibility study identified some issues of variability within the culture results. Internal controls seem to indicate to that sampling procedures and possibly the time to processing are the causes of the disagreement between the culture results from simultaneous samples. These issues should be studied in more detail. We learned several lessons including the following needs: (1) advanced preparation for the operators before the field study starts, including rehearsals of the sampling under different circumstances that resemble the areas where we will execute future studies; (2) use of the same lot of nasal swabs, from the same seller or distributor and stored under adequate conditions before starting the study; (3) registration of lot to which every nasal swab belongs in our sampling forms, in order to assess later if the issue is related to a specific lot or bag of swabs; (4) measure and control the temperature inside each of the storage boxes used in the field to grant an adequate cold temperature that cannot affect the recovery of *Staphylococcus aureus*; and (5) the time for processing the samples must be uniform and should be stated at the beginning of the study, before samples are taken in order to ensure that competing lab projects will not interfere with the sample processing. We propose the following to address these issues:

1. Perform an experimental study in order to assess the sources of variability we identified from our reproducibility study. We will use two different lots of swabs and we will sample two groups of participants, using one of the lots for each group; therefore we will have two samples from each participant and can evaluate if there is a difference in terms of the lots used.

2. Under controlled conditions of temperature, evaluate the effect of the variation of the internal temperature of the storage containers used in the field, to assess which is the best range of temperature that will ensure an adequate recovery of *Staphylococcus aureus*.

3. In the field, all swabs will be stored in boxes with thermometers attached that will show us if there are variations in the temperature as the sampling of the participants takes place variation of the internal temperature of the storage containers used in the field, Therefore, we will take corrective measures to keep the cold conditions in the containers.

4. Link Qa/Qc processes with the proposed repository of isolates such that standardization within the country can be achieved. May need to place this at the Ministry of Health's National Institute if Health or at the Universidad Nacional Mayor de San Marcos.

C. BEHAVIORAL FACTORS

Our study has identified several behavioral factors that affect the performance of the SSTI surveillance system. These can also be extended to any public health effort that is intended to be conducted in the Peruvian Air Force. The poor performance of the SSTI surveillance system not only was caused by the lack of the preferred reporting medium (phone), it was also heavily influenced by the perception of duplicate and useless effort, lack of knowledge regarding the benefit of its utilization and dis-satisfaction with the utilization of the system. We recommend the following be undertaken:

1. Perform studies focused on behavioral patterns of the personnel responsible for the surveillance activities, understanding their motivations (like the use of different types of incentives), barriers, and perceptions about this task. This behavioral research can help us better design strategies that could improve the participation of the stakeholders and improve the report, as well as improving accountability.

2. Understand what are the current practices of health practitioners about the prescription of antibiotics, what is the effect of education on prescription, and the detection of external factors that may contribute to an improper prescription.

D. ENVIRONMENTAL FACTORS

The effect of the geographic location on our results deserves future research initiatives, particularly in Peru where there are a variety of climate conditions, including temperature, precipitation, and altitude. Our study obtained interesting results regarding nasal colonization with *Staphylococcus aureus* that differed depending of the site of

recruitment; and this fact was also observed when we analyzed the prevalence of SSTIs in the different regions of the country. It would be important to conduct:

1. Research studies at border areas, where there is known commercial trade, and the acquisition of imported strains can take place. This research should be a coordinated effort between the adjacent countries.

2. Assessment of the seasonality of nasal colonization with *Staphylococcus aureus* and SSTIs should be addressed in different geographic regions of Peru, determining if the prevalence changes over time based on climate conditions. This information could improve delivery of medical care, developing more efficient guidelines for the use of antibiotics and also improve the supply of antibiotics to each health facility depending on the temporal and geographic variation of the rates.

E. GLOBAL HEALTH EFFORTS

Antimicrobial resistance is a global threat that can impose a heavy burden on public health systems, especially in developing settings where the availability of newer and more potent antibiotics is scarce. Current surveillance efforts are based on the collection of clinical samples from healthcare settings, but do not reflect accurately the situation in the community, which may be the source of cases detected at these hospitals. The sources of information used by international entities must include community measurements instead of solely relying on hospital-based lab surveillance. Among the future efforts we propose are:

1. There should be an international effort to include information from the community level about antimicrobial resistance with the development of sentinel surveillance centers in specific areas where primary care is carried out in order to take samples directly from the community, assessing the etiologic agent and antimicrobial susceptibility. Therefore, we would have a profile of the antimicrobial susceptibility of the community that can serve to improve the medical care of patients at this level.

2. We must adequate the surveillance and lab capabilities of the Peruvian Air Force to the 2005 WHO International Health Regulations. In this way the institution will have a better response facing health threats like antimicrobial resistance.

SPECIFIC RECOMMENDATIONS FOR THE PERUVIAN AIR FORCE

1. Based on our results, MRSA colonization is not a large community issue, but it is necessary to increase the active surveillance of *Staphylococcus aureus* and link this information with that from the SSTIs surveillance system to avoid future problems.

2. Advocacy at the Ministry of Defense for the development and inclusion of biomedical research and disease surveillance as part of the activities in the Directorate of Health of the Peruvian Air Force, partnering with key individuals from the other branches of the Peruvian Armed Forces.

3. Identification of key stakeholders and authorities that can lead to the development of a biomedical research policy, involving them in the design (through workshops with other

military institutions and academic partners who can provide the expertise), policymaking (Directives and rules), needs assessment (available resources, equipment, trained personnel), and identification of best strategies to implement and execute the policy (alliances with academic institutions, development of research capabilities of military personnel).

4. Evaluation of the feasibility and sustainability of biomedical research in the Peruvian Air Force, which must include a long-term projection of the desired capabilities and outcomes. This must include which areas must be prioritized such as epidemiological surveillance, trauma management, health application of mobile technologies in remote areas, etc.; and ultimately, which of these can generate cost savings and position the Peruvian Air Force as a leader in these areas and make sustainable this effort.

5. Improvement of the current epidemiological surveillance policy and procedures following the recommendations of the 2005 WHO International Health Regulations. This must include a thorough assessment of the current situation, identifying pitfalls and proposing corrective measures in order to obtain a more complete surveillance system. Among some of the suggested measures, we would include: Inclusion of the mandatory use of the electronic surveillance system in the current Directive about epidemiologic surveillance, implementation of scheduled monitoring activities in charge of designated personnel, and inclusion of mechanisms for enhancing accountability among the personnel in charge of the surveillance process.

6. Development of an adequate training schedule for the personnel responsible for the surveillance process. This training must be adapted to the rapid turnover of personnel and can include local and distance learning strategies in order to avoid the interruption of the surveillance activities when the stakeholders leave their posts. Additionally, it is necessary to emphasize the use of the full capabilities of the surveillance system, promoting the local analysis of indicators using the datasets available at each health facility.

OVERALL CONCLUSION

Our study has three major conclusions:

1. We identified a low prevalence of baseline nasal colonization with *Staphylococcus aureus* (9.7%) and MRSA (0.3%) in an active duty military population in Peru. However, the prevalence increased over the study period to 20.4% which is consistent with rates published for the region;

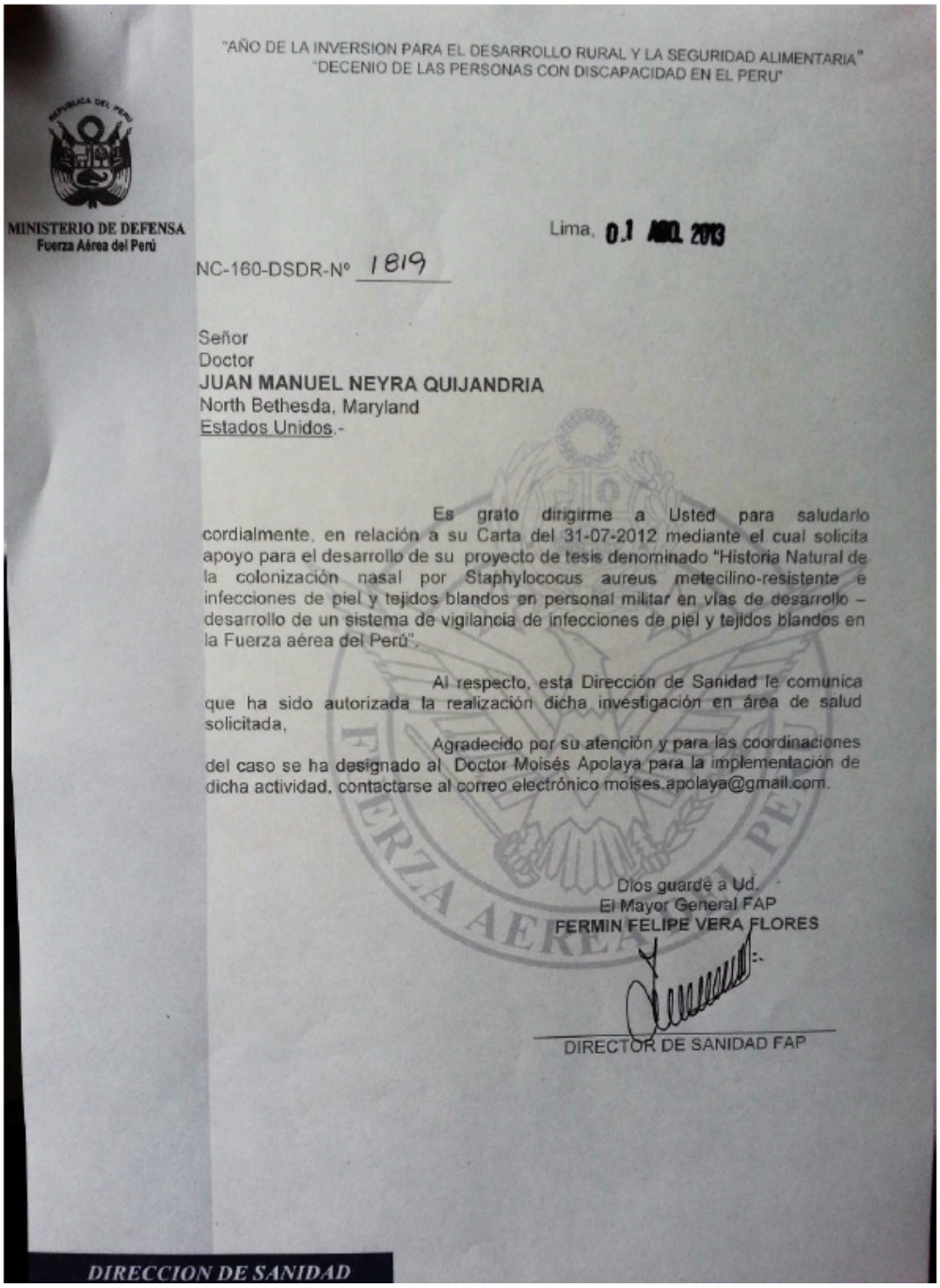
2. We also identified a non-typical community-associated MRSA strain circulating in Arequipa, different from those previously described in the country; and

3. We implemented a SSTI surveillance system with the Peruvian Air Force, but identified several challenges for the adequate functioning of a SSTI electronic surveillance system.

APPENDICES

APPENDIX 1. APPROVAL DOCUMENTS

Appendix 1a. Peruvian Air Force approval



"AÑO DE LA INVERSION PARA EL DESARROLLO RURAL Y LA SEGURIDAD ALIMENTARIA"
"DECENIO DE LAS PERSONAS CON DISCAPACIDAD EN EL PERU"

MINISTERIO DE DEFENSA
Fuerza Aérea del Perú

Lima, 01 AGO. 2013

NC-160-DSDR-Nº 1819

Señor
Doctor
JUAN MANUEL NEYRA QUIJANDRIA
North Bethesda, Maryland
Estados Unidos.-

Es grato dirigirme a Usted para saludarlo cordialmente, en relación a su Carta del 31-07-2012 mediante el cual solicita apoyo para el desarrollo de su proyecto de tesis denominado "Historia Natural de la colonización nasal por Staphylococus aureus metecilino-resistente e infecciones de piel y tejidos blandos en personal militar en vías de desarrollo – desarrollo de un sistema de vigilancia de infecciones de piel y tejidos blandos en la Fuerza aérea del Perú".

Al respecto, esta Dirección de Sanidad le comunica que ha sido autorizada la realización dicha investigación en área de salud solicitada,

Agradecido por su atención y para las coordinaciones del caso se ha designado al Doctor Moisés Apolaya para la implementación de dicha actividad, contactarse al correo electrónico moises.apolaya@gmail.com.

Dios guarde a Ud.
El Mayor General FAP
FERMIN FELIPE VERA FLORES

DIRECTOR DE SANIDAD FAP

DIRECCION DE SANIDAD

Appendix 1b. NAMRU-6 IRB approval

DEPARTMENT OF THE NAVY
U.S. NAVAL MEDICAL RESEARCH UNIT No. 6
3230 LIMA PLACE
WASHINGTON DC, 20521-3230

IN REPLY REFER TO

3900
Ser RS/1106
17 Sept 13

From: Commanding Officer, U.S. Naval Medical Research Unit No. 6
To: Claudio Rocha, MD, Bacteriology Department

Subj: INITIAL APPROVAL OF RESEARCH PROTOCOL NAMRU6.2013.0021 "NASAL COLONIZATION WITH METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN MILITARY PERSONNEL IN DEVELOPING COUNTRY - DEVELOPMENT OF A SKIN AND SOFT TISSUE INFECTION SURVEILLANCE SYSTEM IN THE PERUVIAN AIR FORCE"

Ref: (a) NAMRU-6 INSTRUCTION 3900.6K
(b) Initial Review Application received 14 Aug 13
(c) IRB questions from reviewer panel and PI responses
(d) IRB reviewer panel's final approval on 16 Sept 13
(e) Principal Investigator Responsibilities
(f) BUMED ltr 3900 Ser M00RP/12UM00RP101 of 14 Feb 12

Encl: (1) Protocol, version 03 September 2013
(2) Informed consent form, version 03 September 2013
(3) Enrollment form and follow up form, version 03 September 2013
(4) Log book information form, version 03 September 2013
(5) Report form of skin and soft tissue infections, version 03 September 2013
(6) Presentation about the study

1. In accordance with reference (a), the Institutional Review Board (IRB) panel, reviewed the initial review application under expedited procedures under Category 3 and recommended some modifications to secure approval. The Investigator provided responses to the IRB reviewer panel comments and they were satisfied with them and recommended it for approval. This is a minimal risk study.

2. This study has two primary objectives. First, to determine the natural history of nasal colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) among military personnel in Peru; and second, to develop an epidemiological surveillance process for skin and soft tissue infections (SSTIs) in the Peruvian Air Force through the implementation of reporting of individual events in the current electronic disease surveillance system.

Subj: INITIAL APPROVAL OF RESEARCH PROTOCOL NAMRU6.2013.0021 "NASAL COLONIZATION WITH METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN MILITARY PERSONNEL IN DEVELOPING COUNTRY - DEVELOPMENT OF A SKIN AND SOFT TISSUE INFECTION SURVEILLANCE SYSTEM IN THE PERUVIAN AIR FORCE"

For objective 1, 250 active duty military personnel from the Peruvian Air Force, at each of these four places: Lima, Talara, Arequipa and Iquitos will be enrolled; for objective 2, the surveillance system will be implemented in all the health facilities of the Peruvian Air Force (N = 30), and therefore, the total Peruvian Air Force active military population will be under surveillance.

3. The IRB recommended approval of the research study and verified the following:

- Risk to subjects are minimized and reasonable in relation to anticipated benefits,
- Selection of subjects is equitable,
- Adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data are in place, to the extent legally possible.
- Additional safeguards have been included in the study to protect the rights and welfare of any vulnerable populations; Military personnel will be involved and written informed consent is obtained and documented from each prospective participant.

4. Based on the authority granted to me, I concur with the IRB reviewer panel's recommendation and approve this research protocol.

5. The current approval period for this protocol expires **15 September 2014.** In order for the research to continue without interruption, it is the investigator's responsibility to complete a continuing review report and receive the IRB's recommendation and the Commanding Officer's written approval prior to the expiration. If continuation of the research is not approved by **15 September 2014**, research activities must stop, no new subjects may be screened or enrolled, and analysis of identifiable data may not be conducted.

Subj: INITIAL APPROVAL OF RESEARCH PROTOCOL NAMRU6.2013.0021 "NASAL COLONIZATION WITH METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN MILITARY PERSONNEL IN DEVELOPING COUNTRY - DEVELOPMENT OF A SKIN AND SOFT TISSUE INFECTION SURVEILLANCE SYSTEM IN THE PERUVIAN AIR FORCE"

6. All amendments, including changes to the protocol, such as the addition of new sites or changes in research personnel, and Informed Consent Documents must be prospectively reviewed and approved in writing by the IRB and approved by the Commanding Officer in writing, except where necessary to eliminate apparent immediate hazards to the subjects. Amendments should be submitted using IRB Form 2, Request for Amendment to IRB Approved Research.

7. All related adverse events or unanticipated problems involving risks to subjects or others must be reported via email, telephone, or facsimile to the IRB Chair or Vice-Chairs within 24 hours of learning of the event or problem. The investigator is to submit a written report to the IRB using IRB Form 3, Reporting Unanticipated Problems & Serious Adverse Events, within five (5) business days after you learn of the event of problem.

8. Should this protocol be completed prior to **15 September 2014**, the Principal Investigator is to submit a final report within ninety (90) days after completion. Please note that if approval expires during these 90 days, then a continuing review report must be submitted in time for the IRB to review and provide written approval for continuation prior to the expiration. The Continuing Review Report should be submitted using IRB Form 4, and the Final Review Report should be submitted using IRB Form 5.

9. Points of contact are Dr. Sonia Ampuero, IRB Vice-Chair, at 511-614-4110 or email sonia.ampuero@med.navy.mil, and Ms. Roxana Lescano, Director Research Administration Program, at 511-614-4139 or email roxana.lescano@med.navy.mil

D. B. SERVICE

Copy to:
RAP
DoN HRPP, Washington DC

Appendix 1c. USUHS IRB approval

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
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December 04, 2013

MEMORANDUM FOR JOAN NEYRA, MD, MSc(c), MPH, DrPH(c), PREVENTIVE MEDICINE AND BIOMETRICS

SUBJECT: USUHS IRB #1 (FWA 00001628; DoD Assurance P60001) Approval of Protocol TO-PMB-87-2471 for Human Subjects Participation

Congratulations! The Initial Review for your No More Than Minimal Risk human subjects research protocol TO-PMB-87-2471, entitled "Nasal Colonization with Methicillin-Resistant Staphylococcus Aureus in Military Personnel in a Developing Country - Development of a Skin and Soft Tissue Infections Surveillance System in the Peruvian Air Force" was reviewed and approved for execution on December 04, 2013 by Edmund Howe, M.D., J.D., Chair IRB #1 under the provision of 32 CFR 219.110(b)(1)Suppl.F(3) and 32 CFR 219.110(b)(1)Suppl.F(5). This approval will be reported to the USU IRB #1 scheduled to meet on January 16, 2013.

The purposes of this project are to (1) determine the natural history of nasal colonization with methicillin-resistant Staphylococcus aureus (MRSA) among military personnel in the Peruvian Air Force; and (2) develop an epidemiological surveillance process for skin and soft tissue infections through the implementation of reporting of individual events in the current electronic disease surveillance system. Up to 1000 active duty military personnel (age 18 and over) will be enrolled in the study from the four largest Peruvian Air Force bases (approximately 250 from each base). Participation involves collection of nasal swabs at three time points (baseline, 6months and 1 year) and completion of questionnaires.

The US Naval Medical Research Unit - 6 (NAMRU-6) IRB is the lead IRB and approved this project on September 16, 2013. Approval from the Health Directorate of the Peruvian Air Force was obtained on August 01, 2013.

Authorization to conduct protocol TO-PMB-87-2471 will automatically terminate on December 03, 2014. If you plan to continue data collection or analysis beyond this date, IRB approval for continuation is required. Please submit a USU Form 3204 A/B, application for continuing approval 60 days prior to your termination date. You will receive a reminder from IRBNet.

You are required to submit amendments to this protocol, changes to the informed consent document (if applicable), adverse event reports, and other information pertinent to human research for this project in IRBNet. No changes to this protocol may be implemented prior to

IRB approval. If you have questions regarding this IRB action or questions of a more general nature concerning human participation in research, please contact Micah Stretch at 301-295-0819 or micah.stretch@usuhs.edu.

Learning to Care for Those in Harm's Way

Edmund G. Howe, M.D., J.D.
Chair, IRB #1

Concur / Nonconcur

Arthur L. Kellermann, MD, MPH.
Professor and Dean, School of Medicine

This document has been signed electronically.

"Electronic Signature Notice: In accordance with the "Government Paperwork Elimination Act" (GPEA) (Pub.L. 105-277; codified at 44 USC 3504); Federal and DOD applicable instructions, directives and regulations, documents have been electronically signed and authorized by all who have been required to do so. These signatures have the same effect as their paper-based counterparts. Verification is retained within our protected electronic records and audit trails."

APPENDIX 2. INFORMED CONSENT

"Nasal Colonization with methicillin-resistant *Staphylococcus aureus* in military personnel in a developing country - Development of a skin and soft tissue infections surveillance system in the Peruvian Air Force"

INFORMED CONSENT

PIN #:

Introduction
In recent years there has been an increase of infections, specifically skin and soft tissue infections (tissue located below the skin) by a bacteria called methicillin-resistant *Staphylococcus aureus* (MRSA). This is a bacterium that is usually present (colonizes) in the nose, and can produce severe infections, and it is resistant to some antibiotics (like Methicillin from which this bacterium takes its name). It is present worldwide, especially in people with high mobility, who are considered as the likely source of its dissemination. This bacterium is well known for causing skin infections in young and healthy people who live in enclosed environments, like soldiers and, athletes. Nevertheless, the role of nasal colonization with MRSA in developing settings it is still not well understood.

Purpose
Researchers from the Uniformed Services University, in collaboration with the Peruvian Air Force and the Naval Medical Research Unit No 6 (NAMRU-6) are conducting a study on how frequent is the nasal colonization with MRSA in military personnel. This study is intended to include active duty military members from the Peruvian Air Force. Because you are part of this group, you are being asked to volunteer for this study.

Procedures
If you agree to be in the study, we will provide you with a questionnaire. The questions will help us to know if you have had an infection recently and if you have taken some medications like antibiotics, nasal antibiotics or steroids (anti-inflammatory medication). After you answer the questions, a member of the research team will take one or two nasal swabs. This sample will be tested to determine if MRSA is present in your nose (which indicates that it is colonized with MRSA), the type of MRSA, if it exists, and its susceptibility to some antibiotics.

After this, and at 6 months and 1 year, another one or two nasal swabs will be taken and we will ask you to fill a questionnaire. These samples will be analyzed by our technicians. Therefore, after 1 year of study we will have taken three nasal swabs from you.

Risks
Nasal swab sampling implies no risk except for an immediate, brief and mild feeling of discomfort (as rhinorrhea or sneezing) that disappears in some minutes.
There are no additional risks for you if you take part of this study.

Benefits
There is no direct benefit for you in case you decide to participate in this study. However, being in this research study may help you to understand the risks associated to MRSA colonization. Also, it will help us, as researchers, to understand the behavior and characteristics of this bacterium in the nose, how it can live in your nose without generating disease, the change of the nasal colonization status.

Confidentiality
The test results and your personal information will be kept private to the extent allowed by law. The results of the nasal swabs analysis will be delivered to the Health officer of each of the bases where the samples will be taken. However, a positive result is not indicative of infection, only of colonization, and there is not recommended treatment for nasal colonization. The fact that the bacterium exists in your nose (colonization) will not carry any consequences because neither alter your daily activities nor will affect your job. The involved researchers and the Institutional Review Board may see the results and data from this research. The information from this study may be published but your name and address will not be used.

Compensation / Costs
There will be no cost nor monetary compensation to you for participating in this study.

Voluntary Participation / Withdrawal
Your participation in the study is completely voluntary. You may decide to stop being in the study at any time, even if you agree in the beginning. There will not be any consequences to you for stopping your participation. If you stop being in the study, all your records and your samples will be destroyed.

Future Use
If you agree, the remaining of the nasal swabs that are not used for the tests can be used to look for other infectious diseases in future studies. These remaining samples will not be used for profit, and their use will require your consent and the approval of an IRB. You will not receive any compensation for the use of these samples. If you approve the future use of them, they will be stored with a code only, at the Bacteriology laboratory of NAMRU-6. If you disagree with the future use of these samples, they will be destroyed. The decision about the future use of the remaining samples will not affect your participation in the current study.

☐ I agree with the future use of the remaining samples.

☐ I do not agree. After the completion of this study, I would like the remaining sample to be destroyed.

Contact Person
If you have any questions about this study, or about the future use of your samples, you can call Dr. Joan Neyra at 01-2403161687 (USA), 511-993490725 (Peru) or email to

joan.neyra@usuhs.edu; or Claudio Rocha at 511-971137374 or email: claudio.rocha@med.navy.mil.

Signatures

I agree to participate in this study. You will receive a signed copy of this consent document.

_____
Printed Name

_____
Signature Date

_____
Printed name of person obtaining consent

_____
Signature Date

APPENDIX 3. ENROLLMENT FORM

"Nasal Colonization with methicillin-resistant *Staphylococcus aureus* in military personnel in a developing country - Development of a skin and soft tissue infections surveillance system in the Peruvian Air Force"

I. GENERAL INFORMATION

PIN #:

1. CIP Number:_____

2. Age: _____ years.

3. Gender: □ Female □ Male

4. Rank: _____

5. Today's Date: _____

6. Occupation:
   - Administrative □ Yes □ No
   - Instruction □ Yes □ No
   - Combat Operations □ Yes □ No

II. MEDICAL BACKGROUND

7. In the last 12 months have you been diagnosed, by a doctor, with any of the following medical conditions, such as:

□ asthma □ hypertension □ liver disease
□ chronic bronchitis □ stroke □ kidney disease
□ emphysema □ diabetes □ tuberculosis
□ heart disease □ cancer
□ gastrointestinal disease □ skin conditions □ Malaria

8. In the last 12 months, have you taken any antibiotics?

□ Yes □ No □ Don't know

If yes, specify why you are taking the medication
Specify _____
Specify _____

9. In the last 12 months, have you taken any steroids?

□ Yes □ No □ don't know
Specify _____
Specify _____

10. In the last 12 months, have you used nasal antibiotics?

□ Yes □ No □ don't know
Specify _____
Specify _____

11. In the last 12 months, have you been hospitalized?

□ Yes □ No □ don't know

12. In the last 12 months, have you been diagnosed of skin or soft tissue infections (SSTIs)?

□ Yes □ No □ don't know

13. Have you smoked cigarettes during your life?

□ Yes □ No

14. Did you smoke during the last year?

□ Yes □ No □ no response

15. How many cigarettes in average did you smoke per day during the last year?

..... cigarettes
16. Where do you live?
□ Barracks □ Apartment inside the base
□ Outside the base

17. Since how long are you enrolled in the Force?.....

IV. ADMINISTRATIVE DATA

18. Base: .....

19. Name of Reporter.....
If you have any questions about this study, please contact the study key personnel, Joan Neyra, MD, MPH, at 240-316-1687 or email to joan.neyra@usuhs.edu; or Claudio Rocha at 511-971137374 or email: claudio.rocha@med.navy.mil

APPENDIX 4. FOLLOW-UP FORM OF THE STUDY

"Nasal Colonization with methicillin-resistant *Staphylococcus aureus* in military personnel in a developing country - Development of a skin and soft tissue infections surveillance system in the Peruvian Air Force"

PIN #:

FOLLOW-UP QUESTIONNAIRE

CPI:
Number of nasal swabs taken:

1. **Since you started the follow-up, have you taken any antibiotics?**

 Yes No don't know
If yes, could you specify which?.....

2. **Since you started the follow-up, have you taken corticosteroids?**

 Yes No don't know

3. **Since you started the follow-up, have you participate of deployment missions?**

 Yes No don't know

If you answer yes:
- How many days were you out of the base?.....days
- What were the locations?.....

4. **Since you started the follow-up, have a physician or health care personnel told you that you have a skin and soft tissue infection?**

 Yes No don't know

Report completed by:

If you have any questions about this study, please contact the study key personnel, Joan Neyra, MD, MPH, at 240-316-1687 or email to joan.neyra@usuhs.edu; or Claudio Rocha at 511-971137374 or email: claudio.rocha@med.navy.mil

APPENDIX 5. REPORT FORM OF SKIN AND SOFT TISSUE INFECTIONS

IMMEDIATE REPORT OF SKIN AND SOFT TISSUE INFECTIONS (SSTIs)

1. Date of Admission*:
2. Health facility*:
3. District*:
4. Type of population*:

□ civilian □ officer □ sub-officer
□ troops □ cadet □ alumni

PATIENT

5. Event*: SSTIs
6. ID number:
7. Name
8. Paternal Last Name:
9. Maternal Last Name:
10. Gender*: □ Male □ Female
11. Date of Birth
12. Age*:
13. Address:

PATIENT STATUS

15. How many days ago was the symptom onset*:

16. Is the patient alive?* □ Yes □ No

DEFINED SSTI

17. Type of SSTI*:

□ erysipelas □ impetigo □ folliculitis
□ ecthyma □ furunculosis □ carbunculosis
□ cellulitis □ necrotizing fasciitis

18. Situation*: □ Probable □ Confirmed

19. Check the box if the patient was referred to other Institution: □

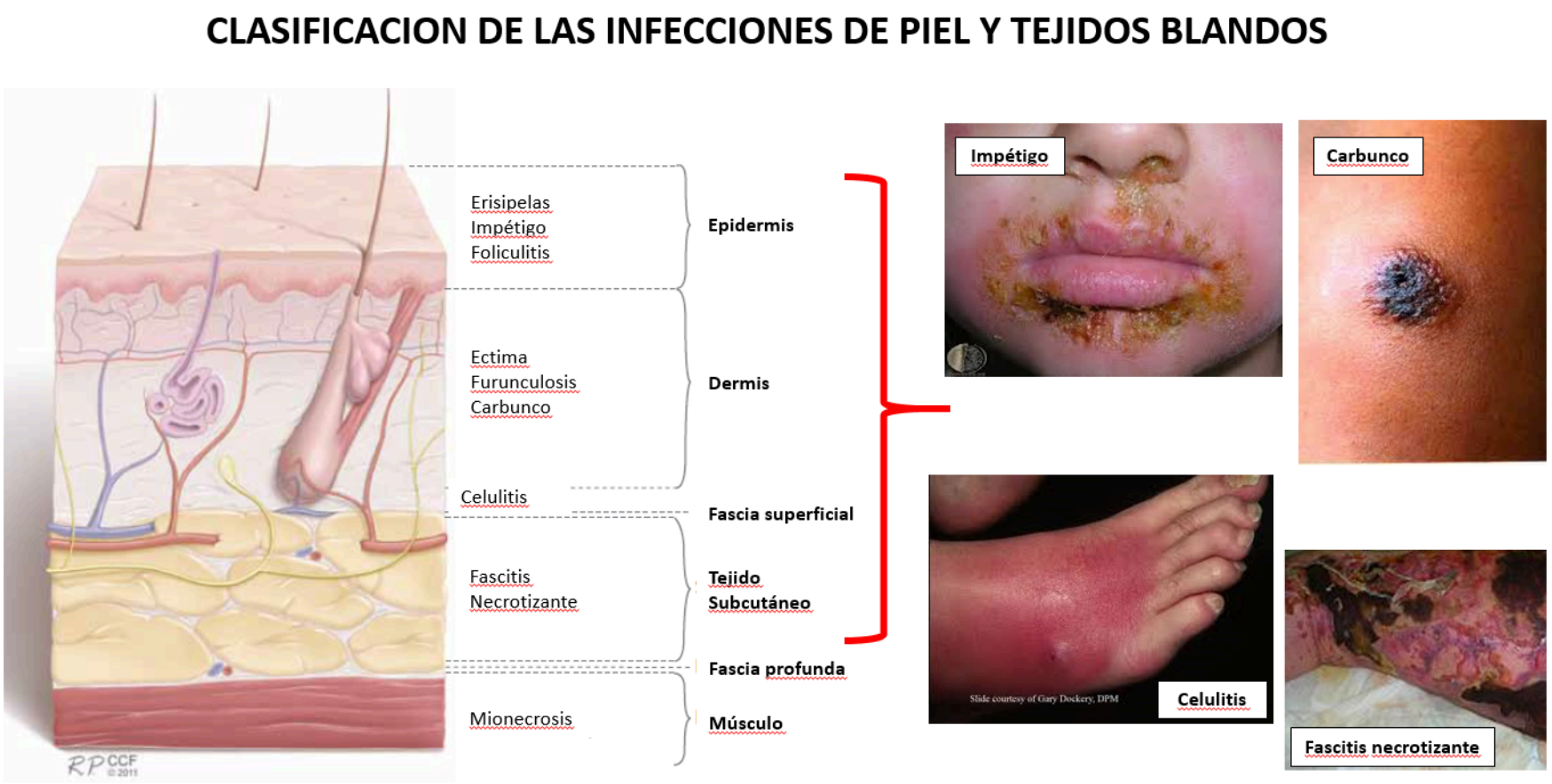
20. Additional Information:

*These items are mandatory and need to be completed for each report.

APPENDIX 6. TRAINING MATERIAL

Appendix 6a. Epidemiologic calendar

Appendix 6b. Definition of SSTIs



Rajan, S. (2012). "Skin and soft-tissue infections: classifying and treating a spectrum." Cleve Clin J Med **79**(1): 57-66.

Appendix 6c. How to report to the electronic surveillance system

SISTEMA ELECTRONICO DE VIGILANCIA EPIDEMIOLOGICA ALERTA DISANFAP

ENFERMEDADES DE REPORTE INDIVIDUAL	
ENFERMEDAD	**COD ALERTA**
REGLAMENTO SANITARIO INTERNACIONAL	
GRIPE HUMANA POR NUEVO SUBTIPO DE VIRU	101
SÍNDROME RESPIRATORIO AGUDO SEVERO	102
VIRUELA	103
POLIOMIELÍTIS POR POLIOVIRUS SALVAJE	104
INMUNOPREVENIBLES	
DIFTERIA	201
FIEBRE AMARILLA SELVÁTICA	202
HEPATITIS	203
MENINGÍTIS TUBERCULOSA MENORES 5 AÑOS	204
PAROTIDÍTIS	205
PARÁLISIS FLÁCCIDA AGUDA (POLIO AGUDA)	206
RUBEÓLA	207
SARAMPIÓN	208
TÉTANOS	209
TOS FERINA	210
VARICELA	211
ZOONOSIS	300
CARBUNCO	301
LEPTOSPIROSIS	302
PESTE	303
RABIA	304
TRANSMITIDAS POR VECTORES	
DENGUE	401
BARTONELOSIS	402
ENFERMEDAD DE CHAGAS	403
LEISHMANIOSIS	404
MALARIA	405
TIFUS EXANTEMÁTICO	406
INFECCIONES CONGÉNITAS	500
SÍFILIS CONGÉNITA	501
ANIMALES PONZOÑOSOS	600
LOXOCELISMO	601
OFIDISMO	602
DAÑOS NO CLASIFICADOS	
BRUCELOSIS	701
CÓLERA	702
GESTANTE VACUNADA INADVERTIDAMENTE	703
INFLUENZA	704
ITS	705
CHANCRO BLANDO	706
HERPES GENITAL	707
GONORREA	708
CLAMIDIASIS	709
SÍFILIS	710
TRICOMONIASIS	711
MENINGITIS MENINGOCÓCICA	712
MUERTE MATERNO-PERINATAL	713
NEUMONÍA	714
SÍNDROME FEBRIL	715
TIFOIDEA	716
TUBERCULOSIS	717
VIH	718
TRAUMA	
AHOGAMIENTO	801
FRACTURAS	802
LESIONES INTERNAS Y PAF	803
QUEMADURAS	804
TRAUMATISMO ENCÉFALO CRANEANO	805

Línea gratuita:

REPORTE POR TELEFONO:

0800 100 70

**Se puede acceder de teléfonos celulares de Movistar

REPORTE POR PÁGINA WEB:

www.vigila.pe/alertadisanfap

CUANDO Y QUE REPORTAR:

Reporte Individual o Inmediato:
Se reporta el **mismo día** que se atiende el caso considerar información demográfica, fecha de inicio de síntomas, NOMBRE y DNI del paciente.

Reporte Colectivo y Consolidado:
Se consolida el número de casos distribuidos por grupos etarios según cada daño en una semana epidemiológica; se reporta al **término de la semana** (Sábado) puede repórtalo hasta el lunes a 1200 horas

Reporte Negativo:
Se notifica **sólo** ante la ausencia de daños que estén bajo vigilancia epidemiológica y se envía cuando concluya la semana epidemiológica.

SEMANA EPIDEMIOLOGICA

Es la unidad de tiempo en vigilancia epidemiológica

JULIO							
SE	D	L	M	M	J	V	S
26							1
27	2	3	4	5	6	7	8
28	9	10	11	12	13	14	15
29	16	17	18	19	20	21	22
30	23	24	25	26	27	28	29
31	30	31					

EJEMPLO: LAS ENFERMEDADES DE REPORTE COLECTIVO SE NOTIFICAN PARA LA SEMANA EPIDEMIOLOGICA 29 (DEL DOMINGO 16 AL SABADO 22): **DESDE EL SABADO A LAS 12:00 HRAS HASTA EL LUNES 24 ANTES DE LAS 12:00 HRS**

empieza el domingo y termina el sábado.

INFORMACION DE CONTACTO

TIP FAP Juan Silvera: RPM: * 172336, * 283902, * 607744

ENFERMEDADES DE REPORTE COLECTIVO	
IRA	1
EDA	2
ASMA - SOB	3
CONTUSIONES	
HERIDAS	
TORCEDURAS O EXGUINCES	

APPENDIX 7. LOG-BOOK INFORMATION FORM

LOG-BOOK INFORMATION FORM

Name of the Base:						
Report completed by:						
Total number of active duty personnel:						
Time Period:						
Subject	**Age (yrs)**	**Gender**	**Rank**	**Date of Diagnosis**	**Diagnosis**	**Therapeutics**
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						

APPENDIX 8. EXECUTIVE SUMMARY FOR THE PERUVIAN AIR FORCE (IN SPANISH)

INFORME DE LOS RESULTADOS DEL ESTUDIO: "Colonización nasal con *Staphylococcus aureus* resistente a meticilina en población militar de un país en vías de desarrollo – Desarrollo e implementación de un sistema de vigilancia de Infecciones de piel y tejidos blandos en la Fuerza Aérea del Perú"

I. RESUMEN

Esta investigación tuvo dos objetivos generales. El primero fue determinar la historia natural de la colonización nasal con *Staphylococcus aureus* resistente a meticilina (MRSA) en personal militar en actividad de un país en vías de desarrollo. El Segundo objetivo general fue desarrollar un sistema de vigilancia epidemiológica de infecciones de piel y tejidos blandos (IPTB) en la Fuerza Aérea del Perú mediante la inclusión del reporte individual de esta enfermedad en el sistema de vigilancia electrónica que la Fuerza Aérea del Perú tiene actualmente.

Para el primer objetivo, realizamos un estudio de cohortes prospectivo en cuatro de las bases mas grandes de la Fuerza Aérea del Perú, recolectando hisopados nasal de 756 miembros en actividad. Las muestras fueron cultivadas para identificar la presencia de *Staphylococcus aureus*, y se determine su perfil de resistencia antimicrobiana. Todas las muestras identificadas como *Staphylococcus aureus* resistente a meticilina fueron

identificados y sometidos a caracterización molecular en los laboratorios de USUHS para determinar su perfil molecular. Para el Segundo objetivo, implementamos la vigilancia de infecciones de piel y tejidos blandos (IPTB) en el sistema de vigilancia epidemiológica que esta funcionando actualmente en la Fuerza Aérea del Perú en sus 27 establecimientos de salud, y luego de 9 meses, evaluamos su rendimiento..

Nuestros hallazgos demostraron que la colonización nasal con *Staphylococcus aureus* al inicio del estudio era menor de lo que esperábamos, pero se incrementó durante el periodo de estudio a niveles consistentes con las tasas publicadas en la región. Este ha sido el primer estudio sobre MRSA en población militar en Latino América y ha determinado en simultaneo las tasas de colonización nasal en 4 diferentes ciudades del Perú. Identificamos un cepa MRSA (similar a la cepa New York/Japan MRSA) que no ha sido identificada previamente en el Perú. Los factores de riesgo para colonización nasal con *Staphylococcus aureus* en esta población son consistentes con aquellos reportados en la literatura. Con respecto a la vigilancia de IPTB, muchos factores que afectaron el rendimiento del sistema de vigilancia. Identificamos numerosos retos para implementar sistemas de vigilancia epidemiológica en países en vías de desarrollo.

En resumen, nuestro estudio encontró una baja prevalencia de colonización nasal con *Staphylococcus aureus* (9.7%) y MRSA (0.3%) en población militar activa en Perú. Sin embargo, la prevalencia se incremento durante el periodo de estudio a 20.4%. Identificamos una cepa MRSA no típica de la comunidad circulando en Arequipa, la cual es diferente a las que han sido previamente reportadas en el país. Implementamos un sistema de vigilancia epidemiológica de IPTB en la Fuerza Aérea del Perú, pero identificamos muchos retos para el adecuado funcionamiento de este sistema.

II. INTRODUCCIÓN

Staphylococcus aureus meticilino-resistente (MRSA) fue documentado por primera vez en 1960, después de la introducción de las penicilinas semisintéticas [1]. Hasta finales de 1990, su presencia se limitaba en gran medida a hospitales con brotes ocasionales. Desde entonces ha habido un aumento sostenido en el número de brotes e infecciones causadas por MRSA, especialmente *Staphylococcus aureus* resistente a meticilina asociada a la comunidad (CA-MRSA).Hay dos subtipos principales de MRSA: asociado a hospitales (HA-MRSA) y asociado a la comunidad (CA-MRSA). Las diferencias entre ellos están relacionadas con la composición genética que confiere la resistencia a los antibióticos, la epidemiología y las manifestaciones clínicas de la enfermedad. Ambas cepas contienen elementos genéticos móviles que confieren resistencia a los antimicrobianos beta-lactámicos. En el caso de HA-MRSA, esta resistencia es adquirida por la presencia de casetes cromosómicos estafilocócicos (SCCmecs I - III), que proporcionan resistencia tanto a beta-lactámicos y a otro antibióticos no beta-lactámicos (multirresistentes). Por otro lado, el CA-MRSA típicamente posee SCCmec tipo IV que sólo confiere resistencia a los antibióticos beta-lactámicos. Aunque menos resistentes a los antimicrobianos, el CA-MRSA posee otros elementos que aumentan su patogenicidad y virulencia. Estos determinantes de virulencia son: Panton-Valentine leucocidina (LPV), la arginina elemento móvil catabólico (ACME) y sobre la expresión de la α-hemolisina.

Los factores de riesgo relacionados con la adquisición de MRSA y los síndromes clínicos resultantes son diferentes dependiendo de la cepa. HA-MRSA se asocia más a las personas ancianas expuestas a atención médica (antecedentes de hospitalización, cirugía, diálisis, residencia de larga duración en centros de cuidado, aislamiento previo de MRSA).

Los síndromes clínicos asociados con HA-MRSA son la neumonía, infección urinaria, infección del sitio quirúrgico, infección del torrente sanguíneo, y la sepsis. Por otro lado, los factores de riesgo descritos para el CA-MRSA incluye a jóvenes saludables, que se encuentran especialmente en ambientes cerrados (atletas, personal militar, presos), la presencia de trastornos de piel y la exposición reciente de antibiótico. Del mismo modo, el CA-MRSA se manifiesta en la piel con más frecuencia que las infecciones de tejidos blandos (SSTI). Seybold et al., encontró que el 87% de toda la piel y tejidos blandos fueron causadas por cepas de CA-MRSA, en su mayoría por USA300, y la infección simultánea de tejidos blandos por esta cepa aumentó las probabilidades de infección del torrente sanguíneo en casi 4 veces. La colonización nasal con CA-MRSA es un factor de riesgo para la infección por el futuro y se correlaciona con la consiguiente bacteriemia por *S. aureus*. Las fosas nasales son el sitio principal de la colonización por *Staphylococcus aureus*. La tasa de colonización varía entre la población: 20% -30% son colonizados persistentemente, el 20% tiene un patrón intermitente de la colonización y el 50% nunca se colonizó. El portador asintomático de *Staphylococcus aureus* en la parte anterior mucosa nasal se considero como el reservorio primario natural de esta bacteria. La colonización nasal se ve facilitada por la anatomía del vestíbulo nasal. La duración de la colonización asintomática es variable. Al parecer, la colonización requiere un cambio en el microambiente de las fosas nasales mediadas por la competencia microbiana, un escenario donde la presencia de factores de virulencia probablemente juegan un papel importante. Al ser un factor de riesgo para la infección posterior por *Staphylococcus aureus*, se debe esperar que las altas tasas de infección debe coincidir con las altas tasas de colonización.

CA-MRSA, está distribuido en todo el mundo y ha sido reportado en diferentes países de Europa y Japón. USA300 es la cepa más común para EE.UU. Esta cepa posee una mayor susceptibilidad a la clindamicina y fluoroquinolonas que otras cepas encontradas en todo el mundo (como los aislados en Rusia [ST5], o en América del Sur y Europa [ST8]); pero la mayoría de las cepas analizadas pertenecen al mismo linaje clonal. Esto tiene implicaciones en el tratamiento especial para el personal militar, teniendo en cuenta que el uso previo de antibióticos tiene un papel en la colonización con CA-MRSA. La distribución de MRSA, especialmente a través de los portadores asintomáticos, puede darse a través de viajes alrededor del mundo. Diversos estudios han demostrado que los viajeros desempeñan un papel en la propagación de MRSA. En viajeros e inmigrantes, la colonización de MRSA se asoció a los viajes a África y Oriente Medio, y fue mayor entre los hombres y en los que viajaban por motivos de trabajo. Sin embargo, las cepas eran similares a las adquiridas en el país de origen. En estudios realizados en los hospitales de combate en Irak, la prevalencia cepa de MRSA USA300 fue en los soldados de combate estadounidenses, mientras que en la población de pacientes fuera de Estados Unidos las tasas de MRSA eran bajos, lo que puede estar relacionado con la colonización previa antes del destaque. En el ámbito militar, las infecciones por *Staphylococcus aureus* complican las lesiones relacionadas con el combate y pueden producir infecciones de la piel y de tejidos blandos durante los destaque o las etapas de formación. La importancia de la colonización nasal y el riesgo real de infección no son claras en el personal militar, debido a que sus actividades suponen múltiples destaques que varían en el tiempo y lugar durante su carrera. Esta exposición a ambientes diferentes puede aumentar la adquisición de MRSA

Las Fuerzas Armadas del Perú implementaron un sistema de vigilancia electrónica de enfermedades infecciosas durante la década pasada. Los reportes recibidos desde cada establecimiento de salud son visualizados en una base de datos en línea, la cual es accesible solo con un numero de usuario y clave. Actualmente el sistema recibe reportes de 50 enfermedades infecciosas, pero las infecciones de piel y tejidos blandos (la enfermedad mas asociada con *Staphylococcus aureus* y MRSA) no estaba incluida en el sistema, a pesar de ser un evento clínico muy asociado a las actividades militares.

En el 2009, se implementó el sistema de vigilancia electrónica de enfermedades infecciosas en la Fuerza Aérea del Perú. Los objetivos del sistema eran estimar tasas de las enfermedades bajo vigilancia, proveer un sistema de detección temprana de brotes, e informa a las autoridades militares acerca de eventos de salud publica. Este sistema cuenta con tres procesos secuenciales: la recolección de datos, el análisis de los datos y la respuesta. Cada paso es ejecutado por diferentes personas. La recolección de datos se inicia en cada base militar donde los reportantes recolectan la información de las enfermedades bajo vigilancia. Luego de esto, el reportante ingresa el reporte al sistema. El análisis de la información la realizan los administradores del sistema e incluye la evaluación del control de calidad de los reportes (determinando al tasa de reporte a tiempo y la tasa de errores de reporte). El monitoreo de las actividades de reporte de cada unidad, y el análisis estadístico de los datos. El proceso final es llevado a cabo por las autoridades de salud, quienes desarrollan guías e implementan las medidas de control pertinentes en respuesta a los eventos reportados.

La falta de información completa sobre las infecciones de piel y tejidos blandos en la población militar activa peruana impide el diseño de medidas preventivas que puedan

reducir el impacto de esta enfermedad, sobretodo en el ambiente militar donde es tan común. Es por ello que es necesario mejorar los indicadores de esta enfermedad, la cual esta relacionada con la colonización nasal con *Staphylococcus aureus*. El propósito de esta investigación fue incrementar el conocimiento sobre colonización nasal con *Staphylococcus aureus*, identificar cepas resistentes (MRSA), determinar las tasas de colonización nasal, y proporcionar un marco para la mejora de la vigilancia de infecciones de piel y tejidos blandos (IPTB) en la población militar activa de la Fuerza Aérea del Perú.

III. Materiales y Métodos

COMPONENTE 1: COLONIZACIÓN NASAL CON *STAPHYLOCOCCUS AUREUS*

Objetivo General

El principal objetivo de este estudio fue determinar la historia natural de la colonización nasal con *Staphylococcus aureus* meticilino-resistente (MRSA) en personal militar en actividad en un país en vías de desarrollo. Para cumplir este objetivo, perseguimos los siguientes objetivos específicos:

5. Calcular las tasas basales de prevalencia de la colonización nasal con *Staphylococcus aureus* y MRSA en la población militar activa de la Fuerza Aérea del Perú.

6. Determinar los factores de riesgo asociados con la colonización nasal con *Staphylococcus aureus*.

7. Determinar el cambio en el estado de colonización nasal y los factores de riesgo asociados con este cambio luego de 6 meses de seguimiento.

8. Determinar el perfil molecular y el genotipo de las muestras resistentes de *S. aureus*.

Diseño de estudio

Se realizó un estudio de cohortes prospectivo. Una cohorte de personal militar en actividad de servicio fue seguida durante un año después de su enrolamiento en el estudio. Después de obtener el consentimiento informado, el participante completo un cuestionario sobre características demográficas y factores de riesgo; y luego se procedió a tomarle hisopados nasales basales para determinar la colonización nasal con *Staphylococcus aureus*, incluyendo MRSA. Las muestras de seguimiento se tomaron a los 6 meses y al año. En estas visitas se le solicito a cada participante que complete un cuestionario de seguimiento y se le tomo nuevos hisopados nasales en cada visita. Cada hisopado nasal fue cultivado y se identifico *Staphylococcus aureus*, y a cada aislamiento positivo se le sometió a pruebas de susceptibilidad antimicrobiana. Los aislamientos resistentes fueron sometidos posteriormente a estudios moleculares para ser tipificados.

Población de estudio

La población de estudio estuvo conformada por personal militar activo destacado en las siguientes bases de la Fuerza Aérea del Perú: GRUP8, ALAR3, GRU11, y GRU42.

COMPONENTE 2: VIGILANCIA DE INFECCIONES DE PIEL YTEJIDOS BLANDOS

Objetivo General

Como parte del estudio, desarrollamos un sistema de vigilancia epidemiológica de infecciones d e piel y tejidos blandos en la Fullera Aérea del Perú, a través de la

implementación del reporte individual de IPTBs en el sistema de vigilancia epidemiológica Alerta-DISAN FAP. Nuestros objetivos específicos fueron:

4. Determinar las tasas de prevalencia de las infecciones de piel y tejidos blandos (IPTBs) reportadas en la Fuerza Aérea del Perú durante el periodo 20012-2014.

5. Incluir el reporte de infecciones de piel y tejidos blandos en el sistema Alerta-DISAN FAP.

6. Evaluar la vigilancia de IPTBs luego de 9 meses de su implementación.

Diseño del estudio

Implementamos el reporte de IPTBs en el sistema Alerta-DISAN FAP en los 27 establecimientos de salud de la Fuerza Aérea del Perú, lo cual nos permitió evaluar la información de la población militar activa de la institución. Luego de 9 meses, evaluamos en sistema de vigilancia de IPTBs. La definición de IPTBs se baso en la profundidad de la infección de las diferentes capas de la piel Definimos IPTBs como todo evento clínico que afecte la epidermis, dermis, fascia superficial y tejido celular subcutáneo. Estas entidades clínicas incluyeron: erisipelas, impétigo y foliculitis (epidermis), ectima, forunculosis y carbunco (dermis), celulitis (fascia superficial) y fasciitis necrotizante (tejido celular subcutáneo)**.**

Para el reporte de estas enfermedades, modificamos un cuestionario previamente validado del sistema de vigilancia Alerta – DISAN FAP. El cuestionario incluyo datos demográficos e información clínica acerca de IPTBs. Luego del diseño del cuestionario, fue incluido en el sistema de vigilancia como reporte inmediato y fue sometido a pruebas antes

de ser puesto a disposición de los reportantes. El entrenamiento de los reportantes se realizó en dos fases: (1) visitas a todas las bases reportantes de la Fuerza Aérea del Perú, y (2) sesiones continuas de reentrenamiento por teléfono o Internet cada trimestre.

Durante la visita inicial a cada unidad reportante, recolectamos todos los casos de IPTBs que estaban registrados en los cuadernos de registro de cada establecimiento durante el periodo 2012-2014. Esto nos permitió calcular las tasas de prevalencia de cada año. Cuando los cuadernos de registro no fueron encontrados, revisamos las recetas medicas

IV. RESULTADOS

OBJETIVO 1: COLONIZACIÓN NASAL CON *STAPHYLOCOCCUS AUREUS*

Población de Estudio

El estudio empezó en Octubre 2013 y se programaron 3 visitas en cada sitio de estudio (Iquitos, Arequipa, Talara and Lima) durante los siguientes periodos: Octubre - Noviembre 2013 (visita inicial), Abril – Agosto 2014 (visita a los 6 meses) y Octubre – Diciembre 2014 (visita al año). La visita inicial se realizó para reclutar a los participantes en cada sitio de estudio (1700 participantes potenciales) y llegar a nuestro tamaño maestral de 1000 participantes. Las visitas de seguimiento a los 6 meses y al año se programaron para tomar las muestras de seguimiento de hisopado nasal a los participantes reclutados.

Reclutamos en total 655 participantes durante la visita inicial y durante la visita de seguimiento a los 6 meses enrolamos a 101 participantes adicionales (Abril – Agosto 2014). Durante la tercera visita de seguimiento no se reclutaron mas participantes. De esta manera reclutamos 756 participantes en total y todos proporcionaron una muestra inicial. De estos

756 participantes, 484 proporcionaron una segunda muestra (35.9% de perdidas al seguimiento) y solo 186 proporcionaron una tercera muestra (71.6% de perdidas al seguimiento).

Las principales razones ara la perdida de participantes durante el periodo de seguimiento fueron retiro de la actividad militar, destaque a otras unidades, ausencias durante las visitas programadas por vacaciones comisiones, y permisos.

Características demográficas

La Fuerza Aérea del Perú cuenta con aproximadamente 10000 miembros en actividad, distribuidos en 4 Alas, una en cada una de las regiones de la Fuerza Aérea a nivel nacional. La edad promedio de los participantes fue 30.3 ± 11.5 años y casi el 60% de ellos era menor de 60 años. El tiempo promedio de servicio al omento del enrolamiento en el estudio fue 10.5 ± 10.9 años y 61% de los participantes tenia menos de 10 años de servicio. 80.7% eran varones y 19.3% eran mujeres.

Los participantes fueron agrupados en 3 categorías: Oficiales, Técnicos y Suboficiales y personal de tropa. Del total de participantes en el estudio, 11.2% eran oficiales, mientras que Técnicos y Suboficiales y el personal de tropa tuvieron similares porcentajes (48.7% and 40.2%, respectivamente). Con respecto al sitio de estudio, Iquitos proporcionó 33.5% de los participantes, mientras que Arequipa y Talara proporcionaron 21% cada uno y Lima, 23.4%.

Características clínicas

152 participantes (20.1%) reportaron tener una condición medica; de ellos, 118 (15.6%) reportaron haber tenido solo 1 enfermedad, mientras que 34 (4.5%) reportaron haber tenido mas de una. 10% reportaron haber tenido una enfermedad gastrointestinal,

5.4% reportaron enfermedades de la piel, 2.6% reportaron enfermedades respiratorias, y 1.1% reportaron haber tenido una enfermedad infecciosa.

Cuando se les pregunto acerca del uso de antibióticos durante el año anterior, 34.8% de los participantes reportó haberlos usado, mientras que 56.5% no lo hizo; y 8.7% no lo sabían o no recordaban. Con respecto al uso de corticoides durante el año previo, 20.2% reportó haberlos usado, 70.9% no los uso, y 8.9% no sabia o no recordaba. 11.6% de los participantes fueron hospitalizados durante el año anterior, 85.3% no fueron hospitalizados y 3% no respondió a esta pregunta. Solo 5.8% de los participantes reportó haber tenido una IPTB durante el año anterior, pero la gran mayoría (89%) no tuvo estas infecciones.

Prevalencia de colonización nasal con *Staphylococcus aureus*

La prevalencia total de colonización nasal (numero total de participantes con al menos una muestra positive para *Staphylococcus aureus* [143] / numero total de participantes) [756]) fue 18.9% (95% IC: 16.1% - 21.7%) durante el periodo de estudio. Nuestra prevalencia total de colonización con MRSA fue 0.3% (2 de 756). Dos participantes tuvieron muestras positivas para MRSA.

***Colonización nasal con Staphylococcus aureus* durante la visita inicial**

La prevalencia de colonización nasal con *Staphylococcus aureus* durante la visita inicial fue 9.7% (n=73, 95% CI: 7.6 – 16.9). La prevalencia fue mayor en aquellos que tenían entre18 a 29 años y 40 a 49 años (10.2% vs. 11.3%); Aquellos con menos de 10 años de servicio tuvieron una prevalencia de 10.6%, similar a la observada en aquellos con mas de 20 años de servicio (9.5%). Cuando se analizo la distribución por genero, los varones

tuvieron prevalencia mas alta que las mujeres (10.3 vs. 6.8%, p=0.201). EL personal de tropa tuvo una prevalencia de colonización nasal de 11.5%, la cual fue mayor a la observada en oficiales y en técnicos y suboficiales (9.5% vs. 8.2%). Talara (4%) tuvo la tasa mas baja de prevalencia de colonización nasal con *Staphylococcus aureus*, mientras que el resto de sitios de estudio tuvo tasas similares (9.1% para Iquitos, 14.0% para Arequipa y 11.3% para Lima).

Cuando se analizaron las condiciones medicas, aquellos que reportaron haber tenido enfermedades respiratorias tuvieron una prevalencia de 30%. Aquellos que usaron antibióticos tuvieron una prevalencia de 11.0%; aquellos que reportaron haber usado corticoides tuvieron una prevalencia de 12.4%; similar a la tasa de aquellos que fueron hospitalizados. Aquellos que reportaron haber tenido IPTB tuvieron una prevalencia de colonización nasal con *Staphylococcus aureus* de 9.1%.

Colonización nasal con Staphylococcus aureus en cada visita

Cuando analizamos la prevalencia de colonización nasal con *Staphylococcus aureus* en cada visita realizada (3 en total), encontramos un incremento en cada visita. Nuestra prevalencia en al visita inicial fue de 9.8%. Durante la segunda visita (6 meses), la tasa de colonización nasal fue 12.4%; y durante la tercera visita (1 año), la prevalencia fue de 20.4%. Ver Figura 1

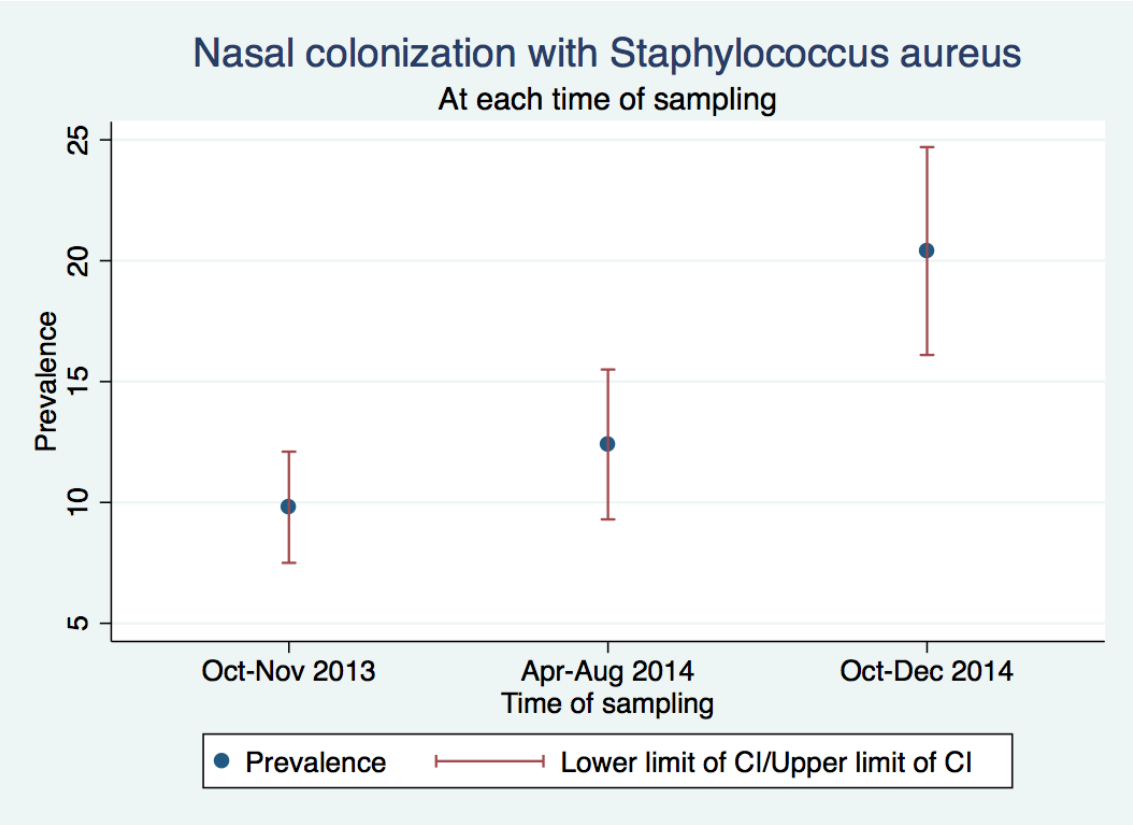


Figura 1. Prevalencia de colonización nasal con *Staphylococcus aureus* en cada visita

Susceptibilidad antimicrobiana de aislamientos positivos y MRSA

Los laboratorios de NAMRU-6 y USUHS procesaron 183 muestras positivas durante el periodo de estudio. Estas muestras mostraron ser sensibles a muchos antibióticos (Ver Tabla 1). Todos fueron susceptibles a ceftaroline, trimetoprim-sulfametoxazol, vancomicina y linezolid. La Resistencia mas alta fue a eritromicina (16.4%), seguida por gentamicina (3.3%). Además, 6.6% presento resistencia inducible a la clindamicina.

Dos muestras (1.1% de las 183 muestras positivas) fueron *Staphylococcus aureus* resistentes a meticilina. Las dos muestras fueron obtenidas en Arequipa. Y fueron tipificadas como PFT USA100, SCCmec tipo II, con características similares a la cepa New York/Japan la cual ha sido reportada en Latino América. Estas dos muestras exhibieron

Resistencia a clorhexidina, no expresaban PVL, ni el gen para la toxina del síndrome de shock toxico (TST). Poseían genes que codifican recombinases, y eran susceptibles a mupirocina.

Tabla 1. Susceptibilidad antimicrobiana de los 183 muestras positivas a *Staphylococcus aureus*

	Numero (%) de muestras		
Antibiótico	**Susceptible**	**Resistente**	**Intermedio**
Clindamicina [a]	179 (97.8)	4 (2.2)	-
Eritromicina	153 (83.6)	30 (16.4)	-
Doxiciclina	180 (98.4)	-	3 (1.6)
Linezolid	183 (100)	-	-
Oxacilina	181 (98.9)	2 (1.1)	-
Rifampicina	183 (100)	-	-
TMP-SMX [b]	183 (100)	-	-
Vancomicina	183 (100)	-	-
Gentamicina	177 (96.7)	6 (3.3)	-
Levofloxacino	182 (99.5)	1 (0.5)	-
Ceftarolina	183 (100)	-	-

a. 6.6% de las muestras exhibió Resistencia inducible a clindamicina
b. TMP-SMX, trimetoprim-sulfametoxazol

Factores de riesgo asociados con la colonización basal con Staphylococcus aureus durante la visita inicial

Los varones tienen 2.4 veces el riesgo de estar colonizados con *Staphylococcus aureus* comparados con las mujeres (95% IC: 1.0 – 5.7, p = 0.043). Estar destacado en cualquiera de los sitios de estudio que no sea Talara también incrementó el riesgo de estar colonizado siendo alta el riesgo en Iquitos (OR: 2.5, 95% IC: 0.9 – 6.5, p = 0.065), Lima (OR: 2.7, 95% IC: 0.9 – 7.3, p = 0.051) y finalmente en Arequipa que tiene un riesgo 4.5 veces mayor (95% IC: 1.7 – 11.9, p = 0.002). El sufrir una enfermedad respiratoria también incrementó el riesgo de estar colonizado, el cual es de 4.5 veces comparado con aquellos

que no reportaron ninguna enfermedad respiratoria (95% IC: 1.4 – 14.7, p = 0.014). Sin embargo, el tiempo de servicio tuvo un mínimo efecto protector (OR: 0.97, 95% IC: 0.94 – 0.99, p = 0.030). Ver Tabla 2.

Tabla 2. Factores de riesgo asociados con la colonización nasal con *Staphylococcus aureus* en la visita inicial

Variable	OR No ajustado	OR Ajustado (95% CI)	Valor p
Uso de antibióticos			
No	Ref.	Ref.	
Si	1.2	1.4 (0.8 – 2.6)	0.283
Hospitalizaciones			
No	Ref.	Ref.	
Si	1.0	1.0 (0.4 – 2.4)	0.980
Diagnostico de IPTBs			
No	Ref.	Ref.	
Si	0.5	0.4 (0.1 – 1.8)	0.237
Sexo			
Femenino	Ref.	Ref.	
Masculino	1.8	2.4 (1.0 – 5.7)	0.043
Sitio de estudio			
Talara	Ref.	Ref.	
Iquitos	2.5	2.5 (0.9 – 6.5)	0.065
Lima	2.8	2.7 (0.9 – 7.3)	0.051
Arequipa	3.9	4.5 (1.7 – 11.9)	0.002
Tabaquismo			
Nunca ha fumado	Ref.	Ref.	
Dejo de fumar	1.7	1.6 (0.8 – 3.5)	0.204
Fuma actualmente	1.2	1.1 (0.6 – 2.1)	0.756
Enfermedades respiratorias			
No	Ref.	Ref.	
Si	3.5	4.5 (1.4 – 14.7)	0.014
Tiempo de servicio	0.99	0.97 (0.94 – 0.99)	0.030

Cambio en el estado de colonización nasal

Luego de 1 año de seguimiento, 484 participantes proporcionaron 2 muestras. Cuando analizamos el cambio en el estado de colonización nasal con *Staphylococcus aureus* en este grupo de participantes con al menos dos muestras, encontramos que la tasa de incidencia fue 11.2% mientras que la tasa de eliminación fue 51%. Ver Tabla 3.

Tabla 3. Cambio en el estado de colonización nasal

		Colonización nasal en la segunda muestra		
		Positivo (%)	**Negativo (%)**	**Total**
Colonización nasal en la primera muestra	**Positivo (%)**	25 (49%)	26 (51%)	51
	Negativo (%)	49 (11.2%)	384 (88.7%)	433
	Total	74	410	484

OBJETIVO 2: VIGILANCIA DE INFECCIONES DE PIEL Y TEJIDOS BLANDOS

Tasas de prevalencia

Recolectamos todos los casos e IPTBs en cada establecimiento de salud de la Fuerza Aérea del Perú desde 2021 al 2014; sin embargo la información solo esta complete para los años 2012 y 2013. Para calcular la prevalencia de IPTBs utilizamos como denominadores números aproximados del total de la población militar activa de la Fuerza Aérea del Perú, estratificada por seo y grado militar.

Durante el periodo 2012 a 2014, se diagnosticaron 1,836 casos de IPTBs (sin embargo, para el año 2014 solo se incluyo información de 25 establecimientos de salud, ya que no pudimos obtener información del Hospital Central y del Hospital Las Palmas). La prevalencia acumulada fue 17%. Las tasas de prevalencia en el año 2012 (7.9%, 95% IC:

[7.4 – 8.4]) y 2013 (5.8% 95% IC: [5.3 – 6.3]) fueron similares. Sin embargo, en el año 2014, la prevalencia fue mucho menor (3.3%, 95% CI: [2.9 – 3.6]).]). Ver Figura 2.

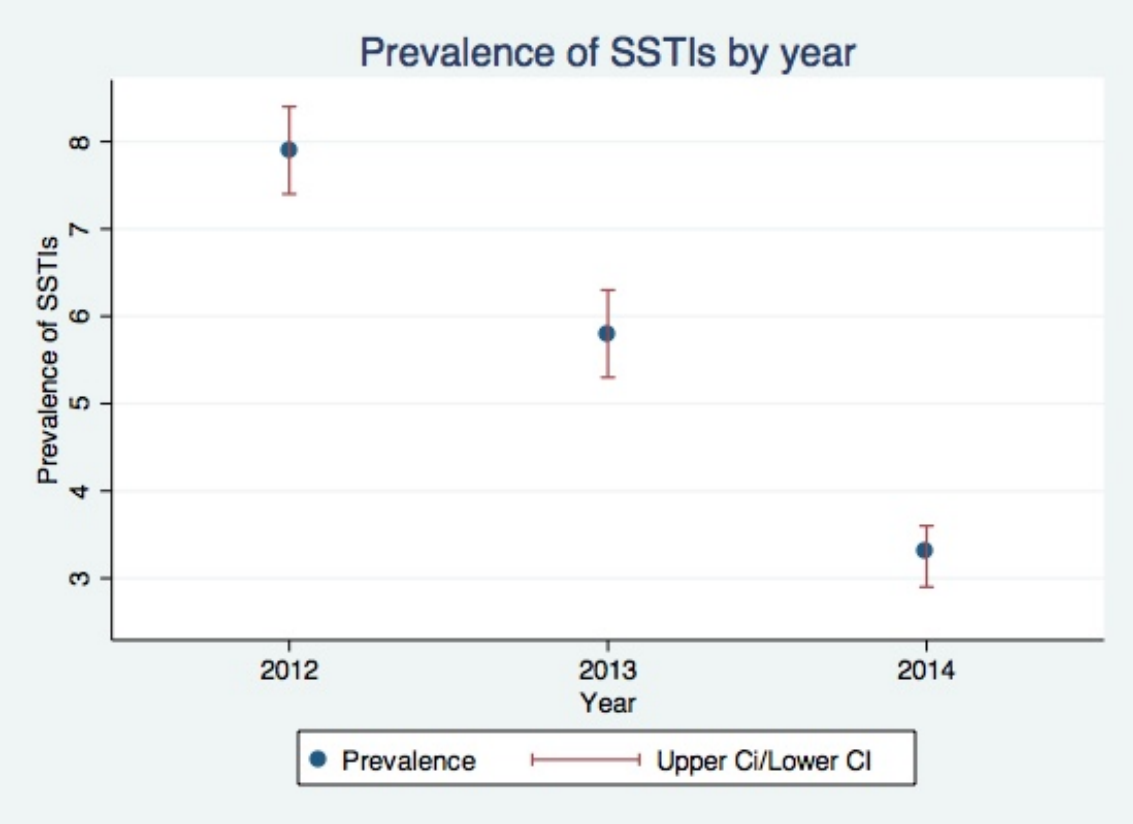


Figure 2. Tasa anuales de prevalencia de IPTBs (2012 – 2014)

La infección de piel y tejidos blandos mas frecuente durante el periodo de estudio fue celulitis (n = 958, 52.2%), seguida por abscesos (n = 434, 23.6%) y piodermitis (n = 219, 11.9%). Ver Tabla 4.

Tabla 4. IPTBs mas diagnosticadas durante el periodo de estudio

Enfermedad	Numero de casos (%) por año			Total
	2012	2013	2014	
Celulitis	408 (48.0)	384 (61.0)	166 (46.6)	958 (52.2)
Abscesos	181 (21.3)	140 (22.2)	113 (31.7)	434 (23.6)
Piodermitis	102 (12.0)	64 (10.2)	53 (14.9)	219 (11.9)
Impetigo	82 (9.6)	27 (4.3)	14 (3.9)	123 (6.7)
Foliculitis	72 (8.5)	7 (1.1)	6 (1.7)	85 (4.6)
Linfadenitis aguda	5 (0.6)	8 (1.3)	4 (1.1)	17 (0.9)
Total	850 (100)	630 (100)	356 (100)	1836 (100)

Evaluación del sistema de vigilancia de IPTBs

Implementación

La Fuerza Aérea del Perú solicito formalmente la incorporación de IPTBs dentro del sistema de vigilancia Alerta – DISAN FAP en Setiembre del 2013. Sin embargo, debido a problemas de tipo técnico, logístico y financiero, la inclusión de este evento se hizo recién a mediados de Marzo del 2014 pero solo para ser reporteado por Internet. Le solicitamos a los reportantes que inicien los reportes de IPTBs desde Abril del 2014. El principal objetivo de esta vigilancia fue obtener estimaciones basales de estas enfermedades, las cuales pueden tener un impacto en la operatividad de las unidades militares.

Las guía de la CDC para evaluar sistemas de vigilancia electrónicos recomienda la determinación tanto de la utilidad del sistema como de los atributos del rendimiento del mismo (simplicidad, flexibilidad, aceptabilidad, calidad de los datos, sensibilidad, oportunidad, estabilidad y representatividad).

Utilidad

Luego de 9 meses, hubo 229 casos de IPTBs en personal militar activo durante el periodo de evaluación (Abril – Diciembre 2014) que debieron haber ido reportados al

sistema de vigilancia. Son embargo, l sistema solo recibió 16 reportes, de los cuales solo 5 correspondían a personal militar activo. De esta manera, solo 2.2% de todos los reportes fueron ingresados al sistema de vigilancia. El sistema de vigilancia de IPTBs no nos permitió determinar las tasas de prevalencia basales de esta enfermedad y comprobamos que no era utilizado durante la visita de evaluación. Cuando se le consulto al personal reportante cuales eran las razones por las cuales no reportaban al sistema, se identificaron 3 casusas principales:

d. ***No hay Internet disponible en su unidad***. El principal medio de reporte es el teléfono, especialmente en unidades localizadas en provincial. Desafortunadamente, esta opción no estuvo disponible para el reporte de IPTBs.

e. ***Falta de personal entrenado para reportar***. Durante la visita de evaluación encontramos que 87.5% de los establecimientos de salud (21 of 25) habían cambiado de personal reportante y no habían sido entrenados en el reporte al sistema.

f. ***Falta de tiempo para enviar el reporte.*** Una de las razones para no enviar los reportes fue que el personal debía dedicarse a otras actividades con mayor prioridad y en algunos casos percibían el reporte al sistema como una actividad repetida ya que también se tenia que reportar al Ministerio de Defensa y al Ministerio de Salud.

Atributos

Simplicidad

La simplicidad del sistema se determine con el análisis de los pasos necesarios para enviar un reporte al sistema. El reporte por Internet de IPTBs requiere llenar un cuestionario de 20 preguntas pero de las cuales solo 11 son obligatorias. Este proceso tomo 3 minutos en ser realizado. De esta manera, el envío del reporte de IPTBs al sistema de vigilancia es simple.

Flexibilidad

Este atributo se midió en base a los cambios requeridos por la Fuerza Aérea del Perú para modificar el sistema de vigilancia de IPTBs. El sistema solo trabajo vía Internet y no se logro incorporar el reporte por teléfono; es así que la flexibilidad es baja.

Calidad de los datos

Solo 1 reporte de los 5 reportes ingresados al sistema estaba registrado en los cuadernos de registro y podía ser comparado contra este. Para este único reporte, la información enviada estaba complete y no tenia errores, pero no podemos inferior nada a partir de un solo registro.

Sensibilidad

Para calcular ala sensibilidad usamos como estándar de oro los casos de IPTBs registrados en los cuadernos de registro durante el periodo de evaluación. La sensibilidad fue de solo 2.2%, muy por debajo del 80% que usualmente se usa como valor limite para una Buena sensibilidad.

Oportunidad

Se solicito a los reportantes que envíen los reportes de IPTBs tan pronto como eran diagnosticados (hasta 1 día después del diagnóstico). Solo pudimos determinar la oportunidad en los 5 reportes ingresados al sistema, y en todos el reporte se había enviado el mismo día del diagnóstico, y por lo tanto los reportes fuero enviados a tiempo.

Aceptabilidad

La aceptabilidad cambio a lo largo del tiempo entre el personal responsable del reporte de IPTBs y las autoridades.

Estabilidad

No pudimos determinar la estabilidad del sistema debido a que no obtuvimos la información técnica sobre el numero de veces que el sistema dejó de funcionar. Sin embargo, en base a reportes verbales del administrador del sistema, esto curio en muy pocas ocasiones.

En resumen, el sistema de vigilancia de IPTBs no funciono como se esperaba. Hubo numerosos problemas desde al implementación tales como el retraso en la inclusión del reporte en el sistema, la falta del reporte por teléfono, la falta de monitoreo de los reportantes por carencia de equipo electrónico para realizar esta tarea, la duplicación de las actividades de vigilancia en las unidades reportantes, la falta de tiempo para enviar el reporte, la movilización del personal entrenado, la falta de entrenamientos programados, entre otros que afectaron el rendimiento del sistema de vigilancia.

V. RECOMENDACIONES ESPECIFICAS PARA LA FUERZA AÉREA DEL PERÚ

7. En base a nuestros resultados, la colonización nasal con MRSA no es un problema extendido en la comunidad, pero es necesario incrementar la vigilancia activa de *Staphylococcus aureus* y relacionar esta información con la que proviene del sistema de vigilancia de IPTBs para evitar futuros problemas.

8. Se debe incluir al Ministerio de Defensa para el desarrollo e inclusión de la investigación biomédica y la vigilancia de enfermedades como parte de las actividades de la Dirección de Salud de la Fuerza Aérea del Perú; formando alianzas con individuos clave de las otras ramas de las Fuerzas Armadas del Perú.

9. Debemos identificar a individuos y autoridades clave que puedan liderar el desarrollo de la política de investigación biomédica, involucrándolos en el diseño (a través de talleres con otras instituciones militares e instituciones académicas que puedan brindar su experticia en el campo), desarrollo de políticas (Directivas y reglamentos), determinación de necesidades (recursos disponibles, equipos, personal entrenado), y en la identificación de alas mejores estrategias para implementar y ejecutar esta política (alianzas con instituciones académicas para el desarrollo de las capacidades de investigación del personal militar).

10. Evaluación de la factibilidad y sostenibilidad de la investigación biomédica en la Fuerza Aérea del Perú, la cual debe incluir una proyección a largo plazo de las capacidades y

resultados que se quieren obtener. Esto debe incluir que áreas deben ser priorizadas tales como vigilancia epidemiológica, manejo de trauma y emergencias, aplicación en salud de tecnologías móviles en áreas remotas, etc.; y finalmente cuales de ellas pueden generar ahorros en los gastos y posicionar a la Fuerza Aérea del Perú como líder en estas áreas y hacer sostenible este esfuerzo.

11. Mejora de la política y procedimientos para la vigilancia epidemiológica en base a las recomendaciones de Reglamento Sanitario Internacional. Esta mejora debe incluir una determinación meticulosa de la situación actual de al vigilancia epidemiológica en la Fuerza Aérea del Perú, identificando problemas y proponiendo medidas correctivas para mejorar el rendimiento del sistema de vigilancia epidemiológica, Algunas de estas medidas podrían ser: (1) Inclusión del uso obligatorio del sistema electrónico Alerta – DISAN FAP en la Directiva actual sobre vigilancia epidemiológica, la implementación de actividades de monitoreo programadas a cargo del personal designado, y la inclusión de mecanismos para mejorar la responsabilidad entre el personal a cargo del proceso de vigilancia epidemiológica.

12. Desarrollo de un adecuado cronograma de entrenamiento para el personal responsable del proceso de vigilancia epidemiológica. Este entrenamiento debe adaptarse a la rápida movilización del personal y puede incluir modalidades de aprendizaje presencial y a distancia para evitar la interrupción de la vigilancia cuando los reportantes dejan sus bases. Adicionalmente, es necesario enfatizar el so de todas las funcionalidades del

sistema de vigilancia, promoviendo el análisis de la información en cada establecimiento de salud,

VI. CONCLUSIONES

Nuestras conclusiones son:

4. Identificamos una baja prevalencia de colonización nasal con *Staphylococcus aureus* (9.7%) y MRSA (0.3%) en población militar activa en el Perú. Sin embargo, la prevalencia se incrementó durante el periodo de estudio a 20.4%;

5. Identificamos una cepa resistente no típica (MRSA) asociada a la comunidad circulando en Arequipa. Esta cepa es diferente a las que se han descrito previamente en el país; e

6. Implementamos un sistema de vigilancia de IPTBs con la Fuerza Aérea del Perú, pero identificamos muchos retos para el funcionamiento adecuado del un sistema de vigilancia electrónico e IPTBs.

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